ETHNOBOTANY

Application of Medicinal Plants

Editors
José L. Martinez
Amner Muñoz-Acevedo
Mahendra Rai

CRC Press
Taylor & Francis Group
A SCIENCE PUBLISHERS BOOK

CPD CERTIFIED
The CPD Certification Service

ETHNOBOTANY

Application of Medicinal Plants

Editors

José L. Martinez
Vice Rectory of Research
Development and Innovation
Universidad de Santiago de Chile
Santiago, Chile

Amner Muñoz-Acevedo
Chemistry and Biology Research Group
Department of Chemistry and Biology
Universidad del Norte
Barranquilla, Colombia

Mahendra Rai
Department of Biotechnology
SGB Amravati University
Amravati, Maharashtra, India

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business
A SCIENCE PUBLISHERS BOOK

Cover credit: Reproduced by kind courtesy of Prof. Amner Muñoz-Acevedo (co-editor)

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2019 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works

Printed on acid-free paper
Version Date: 20180621

International Standard Book Number-13: 978-1-1383-2066-6 (Hardback)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Library of Congress Cataloging-in-Publication Data

Names: Martinez, José L. (José Luis), 1959- editor. | Muñoz-Acevedo, Amner, editor. | Rai, Mahendra, editor.
Title: Ethnobotany : application of medicinal plants / editors, José L. Martinez, Amner Muñoz-Acevedo, Mahendra Rai.
Other titles: Ethnobotany (Martinez)
Description: Boca Raton, FL : CRC Press/Taylor & Francis Group, [2018] | "A Science Publishers book." | Includes bibliographical references and index.
Identifiers: LCCN 2018021988 | ISBN 9781138320666 (hardback : alk. paper)
Subjects: | MESH: Plants, Medicinal | Ethnobotany--methods
Classification: LCC QK99 | NLM QV 766 | DDC 581.6/3--dc23
LC record available at https://lccn.loc.gov/2018021988

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Preface

Ethnobotany is essentially an important branch of plant science, which deals with the study of the relationship between plants and people. Since time immemorial, people are using plants for food, medicine, shelter and agriculture. These plants are mostly used by rural and tribal people for their livelihood. Unfortunately, this traditional knowledge is being lost because it is not being preserved.

The book discusses the current research on ethnobotany and includes the most important investigations of Latin America, Africa, Asia, Egypt, Europe and other parts of the world, where plants are used as medicines by people in general and tribes in particular. In fact, this book incorporates important studies based on ethnobotany of different geographic zones.

More specifically, the book incorporates description about ethnomedicinal plants used in Latin America since the Latin American ethnobotany is less known beyond South America. This book covers medicinal and aromatic plants, ethnopharmacology, bioactive molecules, plants used in cancer, hypertension, disorders of central nervous system, and also as antipsoriatic, antibacterial, antioxidant, antiurolithiatic, etc. Each chapter has been written by one or more specialists in the concerned topic.

The book would be very useful for a diverse group of readers including plant scientists, pharmacologists, clinicians, herbalists, natural therapy experts, chemists, microbiologists, NGOs and those who are interested in traditional therapies. The students should find this book useful and reader friendly.

José L. Martinez
Amner Muñoz-Acevedo
Mahendra Rai

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Contents

Section I

Applications of Ethnomedicinal Plants

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

1

Some Medicinal and Aromatic Plants Used for the Treatment of Cancers/Tumors, with Special Reference to Its Scientific Validations

Amner Muñoz-Acevedo,[1,*] *María C. González,*[1] *Sandra M. Rodríguez,*[2] *Martha Cervantes-Díaz*[3] and *Carlos E. Díaz*[4]

Introduction

Cancer is one of the main diseases causing morbidity/mortality (second cause of death) in the world and the main factor considered for its development/progression/death is heredity, though other factors could also contribute significantly (e.g., behavioral, labor and dietary risks, and viruses). For this reason, a timely cancer diagnosis is required in order to receive the adequate treatment, e.g., radiotherapy, chemotherapy, and surgery, or combination thereof, which are the most frequent therapeutical alternatives. If the cancer is detected and treated early, it has a high cure rate. Based on the statistical data recorded up to 2014, ca. 14 million of people were diagnosed, ca. 8 million of them died, and it is expected to increase ca. 19 million of people diagnosed for 2024. The cancers that produced the most deaths were lung cancer, liver cancer, colorectal cancer, stomach cancer, and breast cancer; it is very important to emphasize that cancer mortality is higher in men than in women (1.4 men/1 woman).

Since 1995 until 2017, the most prestigious multinational pharmaceutical companies (e.g., Novartis, Bristol-Myers Squibb, Boehringer Ingelheim, Bayer HealthCare, etc.) have registered ca. 215 drugs for treatment of cancer and palliative care in the U.S. FDA agency, which has approved them for marketing. Despite this, the pharmaceutical industries are in a search of new drugs for the treatment of cancer, with highest effectiveness, and lowest toxicity and side effects; some active pharmaceutical ingredients

[1] Chemistry and Biology Research Group, Department of Chemistry and Biology, Universidad del Norte, Barranquilla, Colombia.
[2] Economic Analysis Group, Caribbean Economic Studies Institute - Department of Economy, Universidad del Norte, Colombia.
[3] Environmental Research Group for Sustainable Development, Environmental Chemistry Faculty, Universidad Santo Tomás, Bucaramanga, Colombia.
[4] Photochemistry and Photobiology Research Group, Department of Chemistry, Universidad del Atlántico, Barranquilla, Colombia.
* Corresponding author: amnerm@uninorte.edu.co

(f.i., afatinib, belinostat, cabozantinib, eribulin, nivolumab, etc.) obtained by means of chemical synthesis have been effective for treatment of some types of cancers. Besides producing synthetic drugs, some biologically active molecules (e.g., etoposide, teniposide, topotecan, irinotecan, paclitaxel, and vinblastine/vincristine) against cancer have also been isolated from plants (e.g., *Podophyllum peltatum*, *Camptotheca acuminate*, *Taxus brevifolia*, and *Catharanthus roseus*) and approved by FDA for use.

In the scientific literature, numerous articles have been published about plants that have presented cytotoxicity/antiproliferative/antitumor properties on different cell lines, which have proved to be promising. Nevertheless, some of them were not "medicinal plants", or if they were medicinal plants did not have ethnobotanical uses for treatment of cancer; thus, the anticancer activity was the result of "serendipity". In contrast, other medicinal plants that have been used in ethnomedicine for treatment of cancer, when they were tested on cancer cell lines did not have any effectivity. Therefore, after a thorough review of medicinal plants with anticancer/antitumor properties, evidenced in the traditional medicine and scientifically validated, were selected ca. 49 plants, most of them from the ethnobotanical point of view, were useful to treat cancer in general, whose 50% inhibition/lethal/effective concentration/dose values were lower than 10 µg/mL or 10 mM.

Finally, the patent analysis related to the development of anticancer/antitumor drugs from the plants selected allowed to establish that the highest number of patents by plant was for *Solanum lyratum* (56 patents), followed by *Curcuma longa* (54 patents), *Marsdenia tenacissima* (54 patents), *Sarcandra glabra* (44 patents), and *Withania somnifera* (14 patents). Most of these patents were related to the plant fractions/extracts (e.g., *Annona squamosa*, *C. longa*, *M. tenacissima*, etc.) and only some patents with isolated compounds (e.g., saponin mixture from *M. tenacissima*, etc.).

This chapter will discuss some medicinal/aromatic plants ethnobotanically recognized by their efficacy for the treatment of cancers/tumors, whose biological properties (e.g., cytotoxicity, antineoplastic, antiproliferative, and antitumor activities) have been scientifically validated.

Cancer: general aspects

As reported by International Agency for Research on Cancer of World Health Organization in its website (WHO 2015) "cancer is related to a set of diseases" affecting some organs and tissues, in which cells constituting them undergo an alteration/modification in the cell cycle causing them to be divided/replicated without control. The abnormality in cell behavior (cycle altered) is a consequence of changes in genes, e.g., proto-oncogenes (normal cell growth/division), tumor suppressor genes (control cell growth/division) (Levine et al. 1983), and DNA repair genes (fixing damaged DNA), which govern the main cell functions. Therefore, cancer could be the result of a "genetic disease", and in this way, the modified genes could be inherited from parents to children. Likewise, there are other factors (besides the genetic) involved with the development and progression on cancer such as behavioral (tobacco/alcohol abuses, sedentary lifestyles) and dietary (bad food habits) factors, and environmental (chemical substances/physical exposures) risks.

Cancer is one of the main diseases causing morbidity/mortality (second cause of death) in the world: in 2015, ca. 9 million of people died, and the most common cancers that were related to death were lung cancer (~ 1.7 million), liver cancer (788 thousand), colorectal cancer (774 thousand), stomach cancer (754 thousand), and breast cancer (571 thousand) (MEDS/AHRQ 2014). According to the statistical data recorded in 2008–2012, the prevalence of cancer by sickness (new cases) and deceases were ca. 455 new cases/100000 men-women/year and 171 death/100000 men-women/year. In 2014, ca. 14 million of people were diagnosed and it is expected to increase ca. 19 million for 2024. It is worth highlighting that mortality by cancer is higher among men (208 death/100000 men) than women (145 death/100000 women).

Africa, Asia, and Central and South America are the most affected regions of the world by this chronic disease; 70% of deaths occur in the low- and middle-income countries, attributable to that only one in five countries have necessary data and public programs (advertising and prevention) against the cancer. In 2010, the total annual cost of this sickness was estimated ca. USD $ 1.2 trillion, converting it into a disease with a high economic impact for the countries and the world. It is very important to state that the estimated costs did not include the indirect charges: that is, all the required expenditures (family and labor budgets, etc.) for patients to participate in the treatment, which can sometimes equalize the direct

costs (Singleterry 2017). One of the reasons for high economic impact is due to the fact that most drugs available on the market, used for treatment of cancer are very expensive, and these drugs are getting increasingly costlier as they get more complex; also, the costs for patients are related to both the category and the spread of treatment. According to the U.S. National Bureau of Economic Research, between 1995 and 2013 the prices of cancer drugs had an increase of 10% per year. Given the steady rise in prices, the estimates show that payments for drug against cancer could rise from USD $ 100 billion in 2014 to USD $ 150 billion in 2020 (Goldstein et al. 2016).

Figure 1.1 shows the distribution by the number of cancer deaths (2012) in agreement to gender, types of cancer (most common), geographic location and the world, based on data of GLOBOCAN 2012 website (WHO 2015). As can be observed in the figure, there were a significant difference among regions and gender in the number of deaths by types of cancer, f.i., cancers of liver, stomach, and cervical were highly correlated to the region-developing level; in the same sense, 19% of deaths of women due to stomach/liver cancers were in more-developed regions; whilst, 73–81% were in less-developed regions. The highest number of deaths was related to a higher occurrence of lung cancer, mainly in males; however, two specific cancers (breast and cervix) for females were the second cause of mortality. It should be noted that colon-rectal and prostate cancers were persistent (prevailed), regardless of the regions (less-developed/more-developed, except the America-region), indicating the aggressiveness of these types of cancers. For the year 2016 (based on U.S.A. National Cancer Institute website) (NCI 2017) the most common cancers were projected as: breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, kidney and pelvis cancer, leukemia, endometrial cancer, and pancreatic cancer.

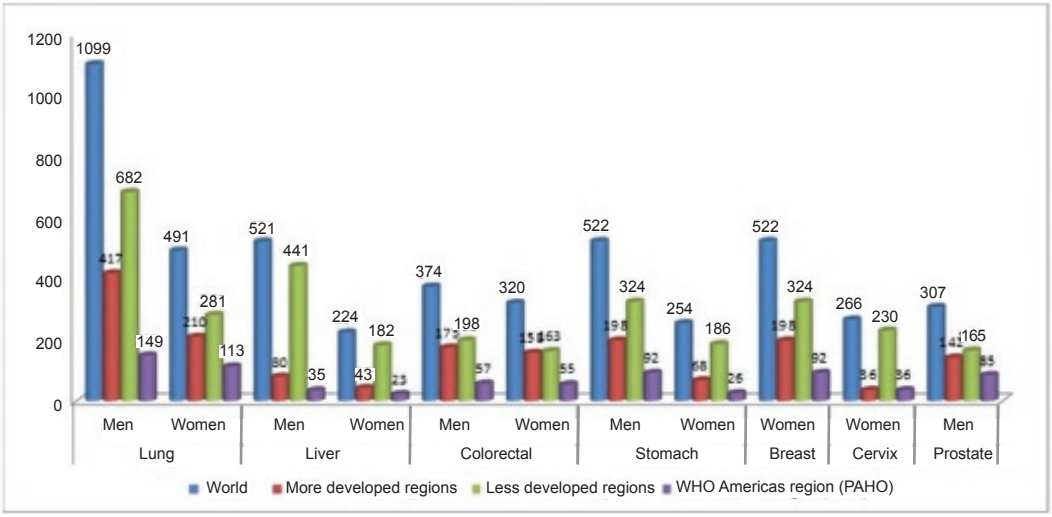


Fig. 1.1

Source: Data were taken of GLOBOCAN 2012 website (WHO 2015). Graph elaborated by authors.

Text mining about cancer, medicinal plants and scientific validation

Through a preliminary review of some medicinal plants/species, whose anticancer/antitumor properties (evidenced in traditional medicine) have been scientifically validated, 157 species/plants were identified; and based on these medicinal plants a search equation was structured into the Scopus database including *title-abs-key*, *doctype and pubyear > 1999*. In correspondence, 2156 registers were found. Frequency of publication monitored, in agreement to the numbers of articles/year concerning individual/specific medicinal plants reported with ethnobotanical information and effectiveness for the treatment of cancers/tumors, together with scientific validation, is shown in the Fig. 1.2. Based on the timeline selected (2000–2017), since 2000 an exponential growth in the register numbers on this theme was observed, 2012–2015 being

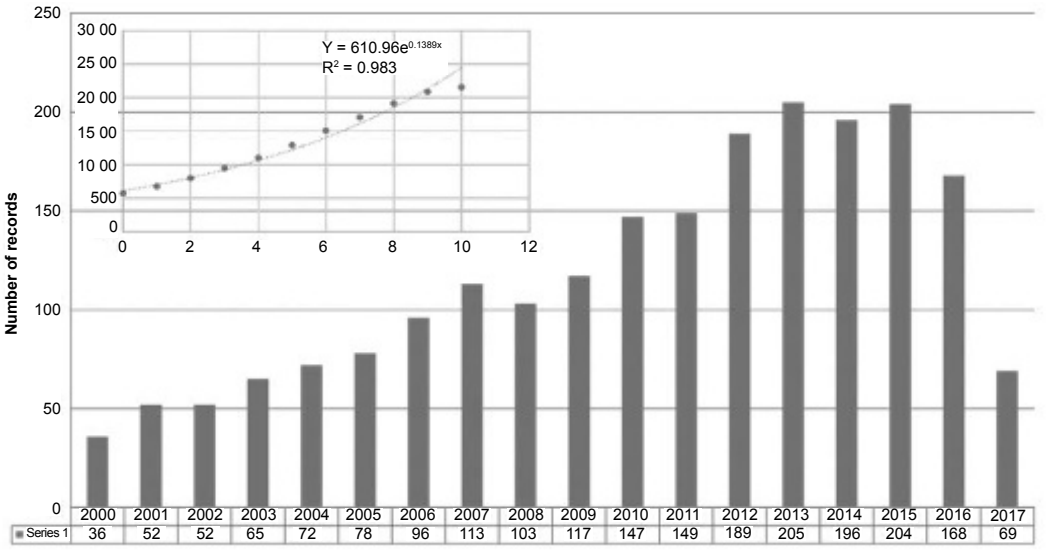


Fig. 1.2

Source: Bibliometry Unit – CRAI-Library, Universidad Santo Tomás (Bucaramanga). Calculations based on Scopus information (Elsevier, B.V., 2017), processed with VantagePoint software (*VP Student, Search Technology*).

the years with the highest article production with 189–204 records. With the aid of De Solla Price's Law[1] (De Solla Price 1963) it was possible to estimate during this period, the growth rate (percentage value/year) of the articles related to this subject, which was 14.9%/year.

Pursuant to the search equation preceding it was also found that the most studied plants related to anticancer properties (against more than five types of cancers) have been *C. longa* (444 articles), *V. album* (428 articles), *A. vera* (134 articles), *C. roseus* (96 articles), *U. dioica* (52 articles), and *A. muricata* (51 articles). In the same way, the main types of cancers examined, in agreement to the numbers of registers found (from highest to lowest), were: breast cancer, colon cancer, lung cancer, prostate cancer, colorectal cancer, etc. with 272 records, 86 records, 76 records, 76 records, 75 records, etc., respectively. A correlation matrix was constructed (Fig. 1.3), based on the previous search equation, to establish the relationships between 157 medicinal plants and the different types of cancers. The figure shows that breast cancer was mainly correlated with *V. album* (101 records), followed by *C. longa* (51 records), and *A. vera* (27 records); similarly, *C. longa* (37 registers), along with *V. album* (13 registers), and *A. vera* (8 registers) were correlated with colon cancer, predominantly.

Traditional drugs used as therapeutical agents against cancer

Since 1995 until 2017, the most recognized multinational pharmaceutical companies have registered ca. 215 drugs for treatment of cancer and palliative care in the U.S. Food and Drug Administration (FDA) agency, who has approved them for use and marketing (FDA 2017). In spite of these achievements, the pharmaceutical industries are in continuous search of new drugs for the treatment of cancer, with highest effectiveness, and lowest toxicity and side effects. Besides producing synthetic and hemisynthetic drugs, some biologically active molecules against cancer have also been isolated from plants and approved by FDA, e.g., etoposide (**1**) and teniposide (**2**) (both compounds isolated from *Podophyllum peltatum* extract) (Damayanthi and Lown 1998) are the active ingredients of two drugs with the trade names of Etopophos®,

[1] De Solla Price's Law results from the observation of the growth in the number of science papers published. As has been observed—with reliable records since the 18th century-, scientific articles have an almost constant growth rate, taking into account both the set of science publications in all disciplines considered as a whole, and the one of each discipline separately.

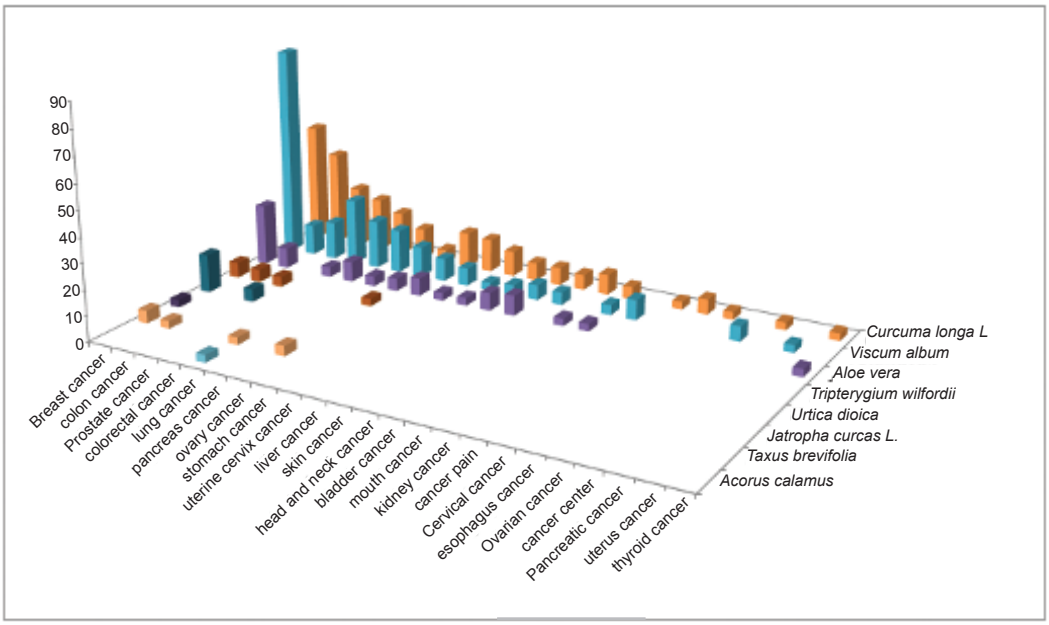
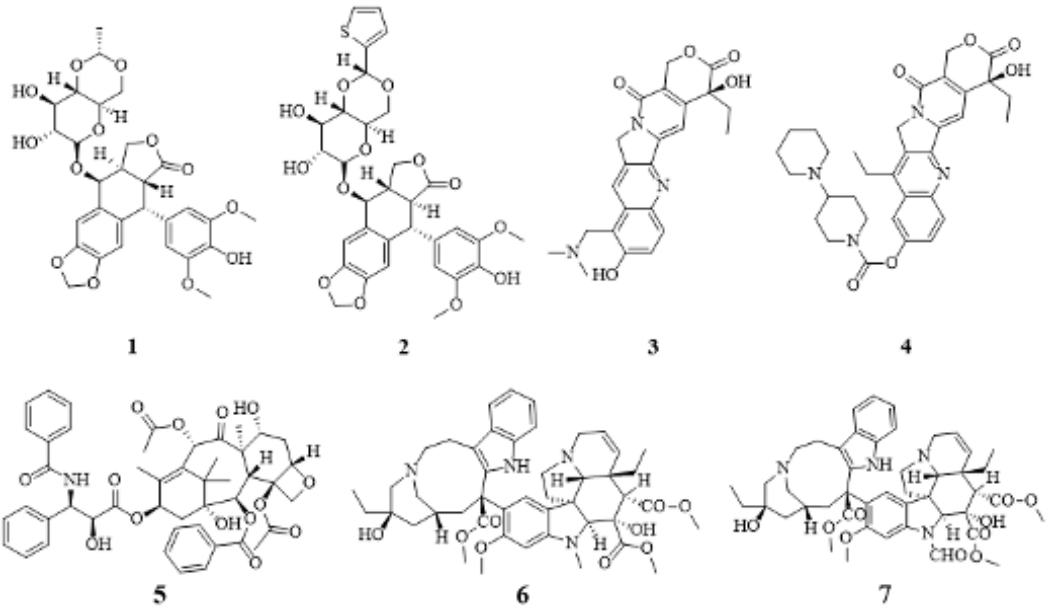


Fig. 1.3

Source: Bibliometry Unit – CRAI-Library, Universidad Santo Tomás (Bucaramanga). Calculations based on Scopus information (Elsevier, B.V, 2017), processed with VantagePoint software (*VP Student, Search Technology*).

and Vumon®, respectively; the camptothecin derivatives, topotecan (**3**) and irinotecan (**4**), isolated from *Camptotheca acuminata* (trade names Hycamptin® and Camptosar®).Two of the most important and successful examples are paclitaxel (**5**) (taxol®) and vinblastine (**6**)/vincristine (**7**) alkaloids (velban®/ oncovin®) (Breza et al. 1975) isolated correspondingly, from *Taxus brevifolia*, and *Catharanthus roseus*. These two drugs are used for treatment of several cancers: taxol® is applied to treat the cancers of breast, lung, cervical, and pancreatic, and sarcoma, etc.; velban®/oncovin® is used for leukemia, neuroblastoma, melanoma, lymphoma, and lung cancer, etc.



Scientific validation of medicinal plants ethnobotanically recognized by their effectiveness for the treatment of cancers/tumors

In the scientific literature, numerous articles have been published about plants that have presented cytotoxicity/antiproliferative/antitumor properties on different cell lines, which have proved to be promising (Réthy et al. 2007, Cates et al. 2013, Ravipati et al. 2013). Nevertheless, some of them were not "medicinal plants", or if they were medicinal plants did not have ethnobotanical uses for treatment of cancer (Mena-Rejón et al. 2009); thus, the anticancer activity was the result of a "serendipity". In contrast, other medicinal plants that have been used in ethnomedicine for treatment of cancer, when these plants were tested on cancer cell lines did not have any effectivity (Alonso-Castro et al. 2011, Schmidt and Cheng 2017). This document dealt with some medicinal plants ethnobotanically recognized by their efficacy for the treatment of cancers/tumors, whose biological properties have been scientifically validated. Table 1.1 contains a list of 49 medicinal plants used for the treatment of cancer/tumor along with forms evaluated of plant preparations and cell lines tested. Additionally, the discussion about anticancer/antitumor properties of plants listed was carried out principally based on type(s) of extract(s) or active compound(s) isolated, cell line(s) tested, assay(s) used, and 50% inhibitory concentration (IC_{50}), 50% lethal dose (LD_{50}), 50% effective dose (ED_{50}), 50% lethal concentration (LC_{50}) or 50% growth inhibition (GI_{50}) values calculated, lower than 10 µg/mL or 10 mM.

Ten diarylheptanoid compounds isolated from *A. japonica* bark were tested on four cell lines. The IC_{50} values obtained by MTT method applied on each cell line were between ~ 17–76 µM for B16, ~ 28–55 µM for SNU1, ~ 48–102 µM for SNU354, and ~ 45–320 µM for SNUC4. In the case of B16 melanoma and SNU1 carcinoma, the most active compounds were **8** (IC_{50} ~ 17 µM) and **9** (IC_{50} ~ 17 µM), respectively. Platyphylloside (**8**) was the compound with lowest IC_{50} values on SNU354 (IC_{50} ~ 48 µM) and SNUC4 (IC_{50} ~ 45 µM) (Choi et al. 2008). One of the main antraquinones [aloe-emodin (**10**)] constituent of *A. vera* was assessed on cell lines of neuroblastoma and Ewing's sarcoma. The neuroectodermal tumor cells were specifically inhibited in growth by **10** and ED_{50} (equivalent to IC_{50}) values were ~ 1 µM, ~ 7 µM, and ~ 13 µM, respectively, for neuroblastoma, pPNET, and Ewing's sarcoma (Pecere et al. 2000). The cytotoxic effects of a solid residue obtained of hydroalcohol extract from *A. scholaris* stem bark were determined on three cell lines. The IC_{50} values were ~ 6 µg/mL (HeLa cells), 10 µg/mL (KB line) and ~ 11 µg/mL (HL60 cells) (Jagetia and Baliga 2016).

A plant studied in almost all continents is *A. muricata*, whose ethnobotany always has been related to antitumoral/anticancer properties. According to a review by Coria-Téllez et al. (2017), six types of acetogenins have been isolated from different parts of the plant; and, these molecules are responsible for the extraordinary effectiveness against cancers. Wu et al. (1995) isolated annomuricins A (**11**) y B (**12**) from plant leaves. These acetogenins showed remarkable antiproliferative capacities when tested on A549 and HT29 cell lines by means of MTT method. The ED_{50} value for **11** was 3.3 x 10^{-1} µg/mL on A549 cells; whilst, for **12**, the ED_{50} values were 1.6 x 10^{-1} µg/mL (on A549 line) and 4.4 x 10^{-1} µg/mL (on HT29 cells). Zeng et al. (1996) isolated annopentocins A-C **13**–**15** and a mixture of *cis*- and *trans*-annomuricin-D-ones (**16**–**17**) from plant leaves. The antineoplastic screening of these compounds was carried out on six cancer lines, resulting in significant effects on all lines; thereby, for **13**, ED_{50} values were between ~ 3.6 x 10^{-2} µg/mL – ~ 18 µg/mL, with PaCa2 line being the most sensitive; for **14** and **15**, ED_{50} values were between ~ 2.1 x 10^{-2} µg/mL – ~ 3.6 µg/mL, being A549 line the most susceptible for both compounds; and for the mixture **16** and **17**, ED_{50} values were between < 10^{-2} µg/mL – ~ 1.3 µg/mL, being the most injured cell lines the A549, HT29 and PaCa2 (ED_{50} < 10^{-2} µg/mL).

To end with the *Annona* species, Li et al. (1990) studied the bark from *A. squamosa*. They isolated three acetogenins (bullatacin **18**, bullatacinone **19** and squamone **20**), which resulted to be highly efficacious against A549, MCF7 and HT29 cell lines (by MTT method). The effectivity order on: (1) HT29 was **18** (< 10^{-12} µg/mL) > **19** (< 10^{-3} µg/mL) > **20** (~ 2 µg/mL); (2) A549 was **18** (~ 9 x 10^{-12} µg/mL) > **19** (~ 1.4 x 10^{-4} µg/mL) > **20** (~ 1 µg/mL); and (3) MCF7 was **19** (< 10^{-7} µg/mL) > **20** (~ 2 µg/mL) > **18** (> 10 µg/mL). Chen et al. (2012) isolated some acetogenins (annosquacins A-D, and annosquatins A and B) from fruit seeds, which were screened to estimate of cytotoxic potential against six cancer cells by MTT method. The six acetogenins were able to powerfully inhibit the growth of all cancer cells, with IC_{50} values between ~ 6 x 10^{-2} µg/mL – ~ 5 µg/mL. The most effective acetogenins with lower IC_{50} values ranged

Table 1.1. Medicinal plant list with ethnobotanical uses for treatment of cancers/tumors (without specificity on a kind of cancer/tumor) along with types of plant preparations evaluated and cell lines tested.

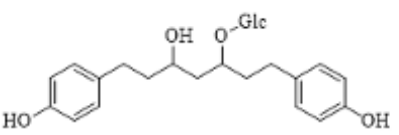
Species	**Ethnobotanical use**	**Ref.**	**Plant preparation form; Type of cell lines**	**Ref.**
Alnus japonica (Thunb.) Steud.	Anticancer	Choi et al. 2008	Isolated compounds (10 diarylheptanoids); B16, SNU1, SNU354 and SNUC4 cell lines.	Choi et al. 2008
Aloe vera (L.) Burm. f.		De Melo et al. 2011	Commercial compound (aloe-emodin **10**), IMR32, IMR5, AF8, SJNPK, pPNET (TC32) and TC106 cell lines.	Pecere et al. 2000
Alstonia scholaris (L.) R. Br.		Ahmed et al. 2001	Solid residue/hydroalcohol extract; HeLa, HL60, and KB cell lines.	Jagetia and Baliga 2016
Annona muricata L.		Gavamukulya et al. 2014, Coria-Téllez et al. 2017	Isolated compounds (acetogenins); PC3, A549, MCF7, A498, HT29 and MIA PaCa2 cell lines.	Wu et al. 1995, Zeng et al. 1996
Annona squamosa L.	Antitumor	Li et al. 1990	Isolated compounds (acetogenins); A549, MCF7, HeLa, HepG2, SMMC7721, MKN45 and HT29 cell lines.	Li et al. 1990, Chen et al. 2012
Aspidosperma polyneuron Mull.	Anticancer	Oliveira et al. 2009	Isolated compound (aspidospermine **21**), HepG2 line cell.	Coatti et al. 2016
Boswellia carteri Birdw.	Antitumor	Zhao et al. 2003	Isolated compounds (boswellic acid acetates); KB, HCT8, B16F10, A2780 and HT1080 cell lines.	Zhao et al. 2003
Calendula officinalis L.	Anticancer	De Melo et al. 2011	Isolated compound [triterpene glycoside compounds (oleananes)]; human cancer cell line panel.	Ukiya et al. 2006, De Melo et al. 2011
Commiphora myrrha Nees	Antitumor	Su et al. 2011	Residual hydroalcohol extract/petroleum ether fraction/isolated compounds; A2780, SKOV3 and Shikawa cell lines.	Su et al. 2011
Cuphea aequipetala Cav.	Anticancer	Vega-Ávila et al. 2004	Acetone:water extract; HCT15 and DU145 cell lines.	Vega-Ávila et al. 2004
Curcuma longa L.	Antitumor	Desai et al. 2008	Isolated curcumin (**28**); CHO and Dalton's lymphoma cell lines. Alcohol extract/isolated **28**; hepatocellular carcinoma.	Kuttan et al. 1985, Naama et al. 2010
Curcuma mangga Valeton & Zijp.	Anticancer	Malek et al. 2011	Hexane/ethyl acetate extracts/isolated compound (2 labdanes); MCF7, KB, A549, Ca Ski, HCT116 and HT29 cell lines.	Malek et al. 2011, Widowati et al. 2013
Davallia divaricata Blume		Monteiro et al. 2014	Isolated compound (davallic acid **31**); A549 cell line.	Cheng et al. 2012
Dendropanax morbifera Lev.		Kim and Song 2011	Isolated compound (**32**); MG63, T98G and Hep3B cell lines.	Lee et al. 2013
Echinops grijisii Hance	Antitumor	Lin and Lin 1993	Isolated tiophenes/bithiophenes; HL60 and K562 cell lines	Zhang et al. 2009

Table 1.1 contd....

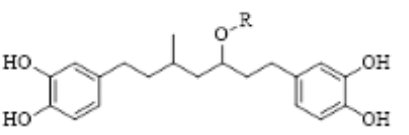
...Table 1.1 contd.

Species	Ethnobotanical use	Ref.	Plant preparation form; Type of cell lines	Ref.
Epipremnum pinnatum (L.) Engl.	Anticancer	Tan et al. 2005	Hexane/chloroform extracts; T47D cell line. Isolated compound (5,6-dihydroxyindole **35**); P388 cell line.	Wong et al. 1996b, Tan et al. 2005, 2007
Erigeron annuus (L.) Pers.		Kim and Song 2011	Hexane/chloroform/50% methanol/aqueous fractions; HeLa, MCF7 and A431 cell lines.	Réthy et al. 2007
Euonymus alatus (Thunb.)	Antitumor	Kim and Song 2011	Methanol extract; SKBR3 cell line	Park et al. 2005
Fissistigma oldhamii Merr.	Anticancer	Wu et al. 1993	Isolated compounds (aporphine alkaloids); A549, HCT8, P388, KB, L1210, CCRFCEM and HL60 cell lines.	Tzeng et al. 1990, Wu et al. 1993
Glochidion zeylanicum (Gaertn.)		Sultana et al. 2014	Aqueous extract; HepG2 and HT29 cell lines.	Sharma et al. 2011
Heterostemma brownii Hayata.	Antitumor	Lin et al. 1997	Isolated compounds (heteromines A/B **39**/**40**); HCE6, Molt4, HL60 and K562 cell lines.	Lin et al. 1997
Himatanthus sucuuba Sp. (M.Arg.)		Perdue and Blomster 1978	Isolated compounds (iridoid lactones); KB cell line and 3T3 cells.	Castillo et al. 2007
Kigelia africana (L.) Benth.		Arkhipov et al. 2014	Methanol/ethanol extracts; SKW3 and MCF7 cell lines, and LLC bearing BDF1 mice.	Momekova et al. 2012
Marsdenia tenacissima (Roxb.) Wight et. Arn.		Luo et al. 1993	Extract/isolated compounds; NSCLC and HepG2/Dox cell lines.	Hu et al. 2008, Han et al. 2012, 2014, 2015
Maytenus blepharodes (Pitt.)	Anticancer	Gupta et al. 1996	Isolated compounds (three phenolic triterpenes); HeLa cell line.	Rodríguez et al. 2005
Maytenus emarginata (Willd.) Ding Hou	Antitumor	Sagwan et al. 2011	Ethanol extract/chloroform/chloroform:methanol/ethyl acetate:acetone fractions/isolated compounds (**49–53**); six cell lines.	Ling et al. 1981, Kuo et al. 1989, 1990, 1994a,b
Melia azadirachta L.	Antitumor	Jain and Tarafder 1970, Paul et al. 2011	Ethyl acetate fraction, ethanol extract/isolated compounds (limonoids); HL60, AZ521, A549, SKBR3 and B16 cell lines. Supercritical extract/three fractions; LNCaP-luc2 and PC3 cell lines. Isolated compounds (mainly nimbolide **69**); N1E115, 143B.TK–, RAW264.7, HeLa, BeWo, HCT116, HT29, CaCo2, U937, HL60, TPH1, MDAMB231, WiDr and B16 cell lines.	Cohen et al. 1996, Roy et al. 2007, Harish Kumar et al. 2009, Priyadarsini et al. 2010, Babykutty et al. 2012, Elumalai et al. 2012, Pan et al. 2014, Takagi et al. 2014, Wu et al. 2014
Nandina domestica Thunb.		Iwasa et al. 2008	Isolated compound (nandsterine **74**); HL60 cell line.	Peng et al. 2014
Panax ginseng C.A. Mey.	Anticancer	Wang et al. 2007, Sultana et al. 2014	Isolated saponins; A549, H1299, H838, H358, LNCaP, PC3, MCF7, MDAMB468, HPAC, Panc1, T98G and A172 cell lines.	Wang et al. 2007
Petiveria alliacea L.		Mena-Rejón et al. 2009	Aqueous decoction; IM9, Daudi, Molt4 and K562 cell lines.	Rossi et al. 1990, 1993, Jovicevic et al. 1993
Pfaffia paniculata Kuntze		Li et al. 2009	Isolated compound (pfaffic acid **78**); B16, HeLa (S3) and LLC lines.	Takemoto et al. 1983

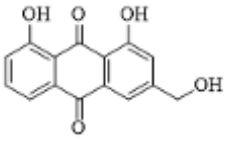
Physalis peruviana L.	Anticancer	Arbiastutie et al. 2017	Ethanol extract; Saos2 and Hep3B cell lines. Isolated compound (4βHWE **79**); H1299 cell line.	Yen et al. 2010, Demir et al. 2014
Rhaphidophora korthalsii Schott		Wong and Tan 1996a	Petroleum ether extract; P388, Molt4, KB and SW620 cell lines.	Wong and Tan 1996a
Rhus javanica L.		Kim and Song 2011	Ethanol extract; Panc1, SW1990 and Capan1 cell lines.	Lau et al. 2008
Rhus verniciflua Stokes		Jung et al. 2007	Hexane fraction; A549, SW620, ACHN and Molt4F cell lines. Isolated compounds (**80**–**85**); A549, SKOV3, SKOV3/PAX, SKMEL2 and HCT15 cell lines. Isolated compounds (**86**–**89**); 29 cell lines.	Hong et al. 1999, Kim et al. 2002, Kim et al. 2015, Choi et al. 2016
Rollinia mucosa (Jacq.) Baill.	Antitumor	Gupta et al. 1996	Chloroform:methanol extract/methanol fraction/isolated compounds (acetogenins **90**–**98**); A549, MCF7, HT29, A498, PC3 and PaCa2 cell lines, and brine-shrimp lethality.	Shi et al. 1996, 1997, Gu et al. 1997, Chávez et al. 1998, 1999, Mata et al. 2001
Sarcandra glabra (Thunb.) Nakai	Anticancer	Tsui and Brown 1996	Isolated compounds (**99**–**101**); HL60 and BGC823 cell lines.	Ni et al. 2013, Wu et al. 2015
Scutellaria baicalensis Georgi	Antitumor	Scheck et al. 2006	DMSO extract; HL60, NB4, THP1, U937, Blin1, Nalm6, Daudi, Raji, Ramos, NCEB1 and NCIH929 cell lines. Isolated compounds (flavones); nine cell lines.	Kumagai et al. 2007, Gao et al. 2008, Huang et al. 2010, Neves et al. 2011, Ji et al. 2015
Selaginella tamariscina (Beauv.) Spring	Anticancer	Kim and Song 2011	Ethyl acetate extract; HeLa, Bel7402 and HT29 cell lines.	Li et al. 2014a
Solanum torvum Swartz.		Arbiastutie et al. 2017	Isolated compound (**105**); A375 and MCF7 cell lines.	Li et al. 2014b, Balachandran et al. 2015
Stephania venosa (Blume) Spreng.			Ethanol extract; NCIH187 cells CH_2Cl_2/butanol fractions/compounds **106**–**111**; HeLa, MDAMB231, MCF7, K562, K562/Adr, GLC4 and GLC4/Adr cell lines.	Niwat et al. 2001, Nantapap et al. 2010, Leewanich et al. 2011, Le et al. 2017
Tabebuia avellanedae Lorentz ex Griseb.		Yamashita et al. 2007, Sultana et al. 2014	Isolated compounds (**112**–**117**); PC3, A549, SiHa and MCF7 cell lines.	Yamashita et al. 2007, 2009, Zhang et al. 2015
Tabebuia rosea (Bertol.) DC.		Hemamalini et al. 2012	Isolated compounds (**112**, **118**–**121**); KB and HeLa cell lines.	Sichaem et al. 2012
Tripterygium wilfordii Hook. f.		Chen et al. 2008, Yang et al. 2011	Isolated compounds (**122**–**131**); HepG2, Hep3B, Bcap37, U251, MCF7, HeLa, A549 and A549T cell lines.	Yang et al. 2011, Gao et al. 2016, 2017, Fan et al. 2016
Viscum album L.		Kim and Song 2011	Aqueous extract; SKBR3 cell line.	Weissenstein et al. 2016
Withania somnifera L. Dunal.		Sultana et al. 2014	Hydroalcohol extract; A549, MCF7 and PA1 cell lines. Methanol extract/isolated compounds (**132**–**135**); NCIH460, HCT116, SF268 and MCF7 cell lines.	Jayaprakasam et al. 2003, Nema et al. 2013, Choudhary et al. 2015



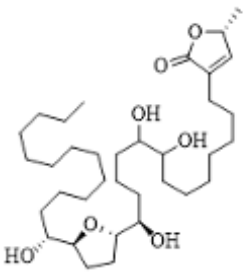
8



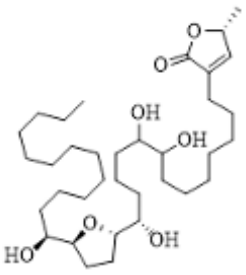
9 - Xyl$^{3}\!\!-\!\!^{1}$ Glc



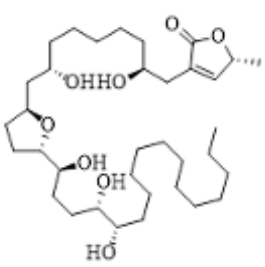
10



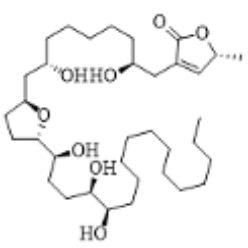
11



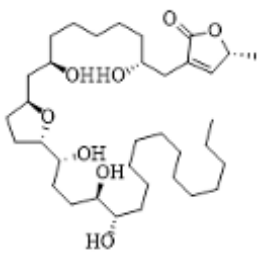
12



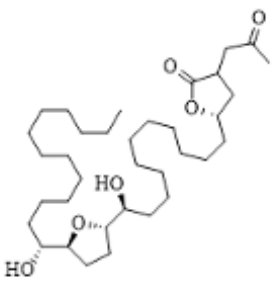
13



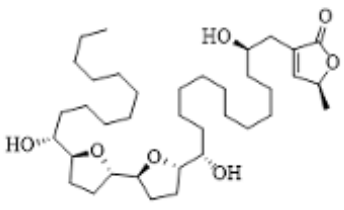
14



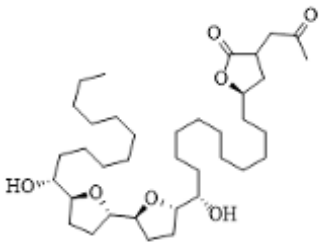
15



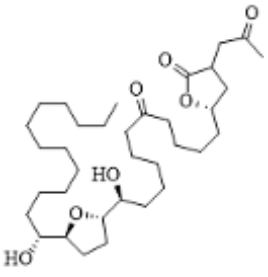
16,17



18



19



20

among ~ 6 x 10^{-2} µg/mL – ~ 5 x 10^{-1} µg/mL, and ~ 9 x 10^{-2} µg/mL – ~ 3 x 10^{-1} µg/mL, were annosquatin A and annosquacin C, in that order. In the same sense, annosquatin B and annosquacin B were secondary, with IC_{50} values between ~ 8 x 10^{-2} µg/mL – ~ 2 µg/mL and ~ 1 x 10^{-1} µg/mL – ~ 3 µg/mL, respectively; and lastly, annosquacins A and D had IC_{50} values among ~ 4 x 10^{-1} µg/mL – ~ 4 µg/mL and ~ 3 x 10^{-1} µg/mL – ~ 5 µg/mL, correspondingly. The most active compounds per each cell line were annosquatin B (IC_{50}: ~ 8 x 10^{-2} µg/mL) on A549 cells, annosquacin C (IC_{50}: ~ 3 x 10^{-1} µg/mL, ~ 1 x 10^{-1} µg/mL) on HeLa/HepG2, and SMMC7721 cancer lines; annosquatin A (IC_{50}: ~ 6 x 10^{-2} µg/mL, ~ 1 x 10^{-1} µg/mL) on MCF7 and MKN45 cell lines.

From *A. polyneuron* roots an indole alkaloid (aspidospermine **21**) was isolated which was tested on HepG2 cells. A concentration of 75 µM of **21** was able to decrease *ca.* 68% of cell population (Coatti et

al. 2016). Zhao et al. (2003) assayed an isoform mixture [α-(**22**) and β-(**23**)] of boswellic acid acetate (isolated from *B. carteri* resin) on five cancer lines. According to the authors, the compound mixture (**22** and **23**) showed a growth potential inhibition on KB, HCT8, and A2780 cells with IC_{50} values between ~ 11 μM – ~ 13 μM.

The other well-known plant is *C. officinalis*, where there are reports on its use for cancer treatment. One of these reports was prepared by Ukiya et al. (2006), where they isolated two triterpene glycosides (**24** and **25**), from the flowers. Compounds **24** and **25** were tested (by SRB method) on a panel of 60 human cancer cell lines (derived of seven types of cancer: lung, colon, melanoma, renal, ovarian, brain and leukemia). The authors found that **24** showed GI_{50} values < 10 μM against almost of the cancer cells tried except for leukemia (CCRF-CEM cells), renal (CAKI-1 and UO-31 lines) and breast (NCI/ADR-RES cells) cancer lines. In addition, **24** showed the most potent cytotoxicity against leukemia (Molt4 and RPMI8226 lines), colon (HCC2998 cells), and melanoma (LOX IMVI, SKMEL5 and UACC62 lines) cells, with GI_{50} values of ~ 0.8 μM – ~ 1.0 μM. Compound **25** exhibited GI_{50} values < 20 μM against all of the cancer cells tested, except for ovarian (IGROV1 cells) and renal (UO31 cells) lines.

A famous herb is Myrrh (*C. myrrha*, Burseraceae), whose multi-purpose fragrant resin has been used millennially. Su et al. (2011) reported the evaluation of cytotoxicity effects (by MTT method) against gynecologic cancer cell lines (A2780, SKOV3, SiHa and Shikawa) of residual hydroalcohol (85%) extract (RHE), Petroleum Ether Fraction (PEF) and two isolated compounds (**26** and **27**) from *C. myrrha* dried resin. **26** and **27** significantly inhibited the growth of three cell lines; the IC_{50} values were between ~ 47 μM – ~ 64 μM for **26** and ~ 27 μM – ~ 36 μM for PEF. The most susceptible lines were A2780 (~ 47 μM) for **26**, and SKVO3 (~ 27 μM) for **27**. A plant used in traditional Mexican medicine, as treatment of different types of tumors since the prehispanic ages is *C. aequipetala* (Lythraceae). Vega-Ávila et al. (2004) studied the cytotoxic potential (ED_{50}) of different fractions from acetone:water (7:3) extract of the whole plant, using the SRB assay and two cell lines. The ED_{50} values were ~ 8 μg/mL (DU145 cells) and ~ 19 μg/mL (HCT15 line) for total extract in each cell line. E (yellow powder, constituted by compounds type flavonoids) and PB1 (solid with a metallic appearance, constituted by compounds type tannins) fractions, from total extract, presented ED_{50} values of ~ 0.4 μg/mL and ~ 2.5 μg/mL, respectively.

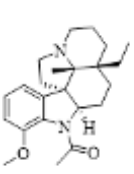
The rhizome of *C. longa* (Zingiberaceae), popularly known as turmeric or Indian saffron, has been applied for cancer prevention/treatment. Kuttan et al. (1985) evaluated *in vitro* (by using cell culture of Chinese Hamster Ovary (CHO) line and normal lymphocytes) the anticancer activity of the isolated curcumin (**28**) from turmeric rhizome; in a similar manner, 8 μg/mL of **28** showed an effective cytotoxicity (100% cell death) for normal lymphocytes, and human leukemic cells; while 4 μg/mL of **28** produced the 100% death on Dalton's lymphoma cells. The *in vitro* experiments indicated that curcumin was cytotoxic at concentrations between l μg/mL – 4 μg/mL. Also, Naama et al. (2010) studied the rhizome extract and isolated curcumin from *C. longa* of Iraqi origin. The cytotoxic effect of alcohol extract/curcumin from *C. longa* was valued against the hepatocellular carcinoma by means of MTT assay. The IC_{50} values were 0.8 μg/mL and 0.6 μg/mL for total extract and isolated **28**, respectively. Other authors have studied the antineoplastic effect of **28** against different types of cancer/tumor, e.g., breast carcinoma cells, colorectal cancer cells, gastric cancer, leukemic cell lines, lung squamous cell carcinoma, ovarian cancer cells (Shao et al. 2002, Shi et al. 2006, Alaikov et al. 2007, Guo et al. 2013, Zhao et al. 2015, Zhou et al. 2017).

C. mangga from Indonesia is a species closely related to *C. longa*. Malek et al. (2011) investigated the cytotoxic effects of the methanol extract and the hexane/ethyl acetate fractions from *C. mangga* rhizome against six cell lines by using SRB assay. The authors found that methanol extract and the two fractions showed good cytotoxic effects against the cell lines, whose IC_{50} values were between 8.1 ± 0.2 μg/mL and 47.1 ± 0.5 μg/mL. The hexane fraction showed the best antineoplastic effects against the six cancer lines, with IC_{50} values among 8.1 ± 0.1 μg/mL (MCF7 line) and 31.5 ± 0.1 μg/mL. Likewise, they isolated to *E*-labda-8(17),12-dien-15,16-dial (**29**) and zerumin A (**30**), which displayed high cytotoxicity on six cancer lines. **29** presented IC_{50} values between ~ 4 ± 1 μg/mL (MCF7 line) – 19.9 ± 0.4 μg/mL; whilst for **30**, the IC_{50} values were among 8.7 ± 0.3 μg/mL (MCF7 line) – 16.2 ± 0.2 μg/mL (Widowati et al. 2013).

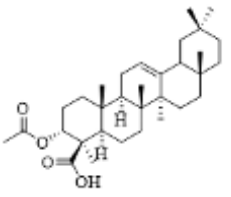
D. divaricata (Davalliaceae) is a species from Taiwan, which has been traditionally applied as a treatment for lung cancer. Cheng et al. (2012) isolated the davallic acid (**31**) from plant dried rhizome and evaluated the ability of **31** to inhibit the growth of A549 cells using MTT test. They found that 20 μM of **31** decreased A549 cell viability up to 73% (12 hours) and 57% (24 hours). *D. morbifera* (Araliaceae) is

an endemic species growing in South Korea and has been used in traditional medicine. Lee et al. (2013) isolated dendropanoxide (**32**) from plant dried leaves and assessed the capability of compound **32** to inhibit the growth of three types of cell lines using MTT assay. The results showed that 40 µM, 50 µM and 60 µM of compound **32** inhibited the cell growth up to ~ 55% , ~ 60% and ~ 60% on Hep3B, T98G and MG63 cell lines, respectively.

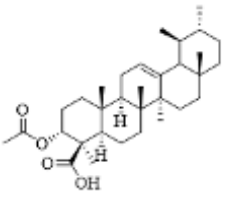
E. grijisii (Compositae) is a species broadly distributed in China, and it is listed in "Chinese Pharmacopeia" by its different ethnobotanical uses. Zhang et al. (2009) investigated the cytotoxicity against two human leukemia cell lines, by using modified SRB method, of seven compounds type "thiophenes/ bitiophenes" isolated from *E. grijisii* roots. The authors found that all compounds exhibited cytotoxic effects against the cell lines examined, with IC_{50} values between ~ 0.2 µg/mL and ~ 17 µg/mL for HL60 cells and ~ 0.4 µg/mL and ~ 31 µg/mL for K562 cells. The most cytotoxic compound were **33** (IC_{50}~ 0.2 µg/ mL and ~ 0.5 µg/mL) and **34** (IC_{50}~ 0.3 µg/mL and ~ 0.4 µg/mL) for the two cell lines (HL60 and K562). *E. pinnatum* (Araceae, common name: Dragon tail plant) has been widely used in Malaysia and Singapore as a traditional anticancer preparation. Anticancer properties of chloroform/hexane extracts from whole plants were validated by Tan et al. (2005, 2007) against T47D line using MTS/PMS assay. Thus, the IG_{50} values were ~ 12 µg/mL (48 hours) and ~ 6 µg/mL (72 hours) for chloroform extract; the IG_{50} values were ~ 12 µg/mL (48 hours) and ~ 3 µg/mL (72 hours) for hexane extract. Also, Wong et al. (1996b) isolated **35** from *R. korthalsii* leaves, and determined its antineoplastic capacity against P388 cancer line by MTT assay. The IC_{50} value calculated for **35** was of ~ 4 µg/mL.



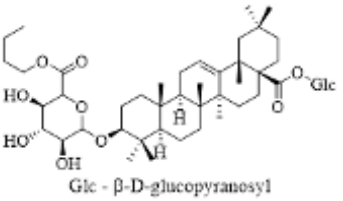
21



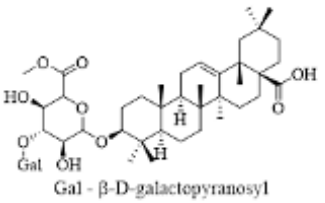
22



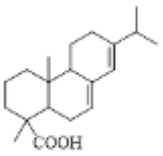
23



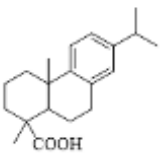
24



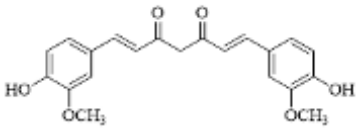
25



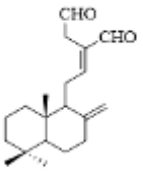
26



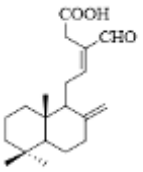
27



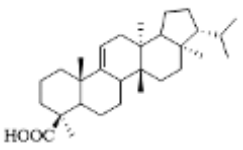
28



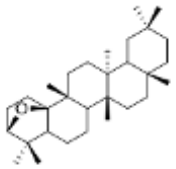
29



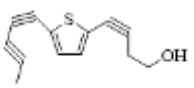
30



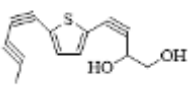
31



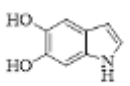
32



33



34



35

Regarding *E. annuus* (Asteraceae), Réthy et al. (2007) screened the *in vitro* antiproliferative activity of four types of fractions/extracts from this plant against three cell lines, using the MTT assay. Inhibition percentage values, from plant root extracts on MCF7 line,were ~ 14 ± 3% – ~ 62 ± 2%, when 10 µg/mL of each extracts/fractions were tested. The IC_{50} values calculated for hexane and chloroform fractions against the three cell lines were ~ 12–13 µg/mL (HeLa line), ~ 6–9 µg/mL (MCF7 line) and ~ 13–20 µg/mL (A431 line). A traditional Korean medicinal plant used for centuries to treat tumors in Korea and China is *E. alatus* (Celastraceae). Park et al. (2005) evaluated the antiproliferative and apoptotic capacities of methanol extract from plant on SKBR3 cell line by MTT method; the ED_{50} value was of 6.5 ± 0.3 µg/mL and the methanol extract induced apoptosis on cancer cells.

F. oldhamii (Annonaceae) is a species used in folk medicine for treatment of tumors in Taiwan and southern China. Wu et al. (1993) demonstrated the cytotoxicity of xylopine (**36**) and norannuradhapurine (**37**) isolated from plant against four cell lines, by MTT assay. The ED_{50} values for **36** and **37** were among ~ 2 µg/mL and ~ 3 µg/mL for the four cell lines. Even so, the most sensitive cells were KB and HCT8 to compounds **36** and **37**, whose ED_{50} values were ~ 2 µg/mL and ~ 3 µg/mL, independently. Tzeng et al. (1990) evaluated the inhibitory effect on growth of three leukemic cell lines, using the MTT method, of aporphine alkaloids (**37** and fissoldine **38**) isolated from the plant. The IC_{50} values of **37** and **38** were ranged between ~ 3–15 µg/mL (~ 9–35 µM) for the three cell lines. Nonetheless, L1210 and CCRFCEM lines were the most susceptible cells to alkaloid **37** with IC_{50} values of ~ 4 µg/mL (~ 9 µM) and ~ 6 µg/mL (~ 14 µM), correspondingly; whilst the IC_{50} values of **38** was of 15 µg/mL (~ 35 µM) on HL60 cells.

In the traditional ayurdevic medicine from India, there is a plant called Neeru mamidi [*G. zeylanicum* (Euphorbiaceae)], which has been used for the treatment of cancer. Sharma et al. (2011) evaluated the *in vitro* cytotoxic activity of aqueous extract from plant roots against two cell lines, by using the XTT method. The CC_{50} values were ~ 3 µg/mL and ~ 26 µg/mL, for the HepG2 and HT29 cell lines, respectively. Based on the folk medicine of Taiwan about the use for treatment of tumors from *H. brownii* (Asclepiadaceae) aerial parts, Lin et al. (1997) isolated heteromines A (**39**) and B (**40**), which were evaluated against four cell lines by using the MTT assay. The IC_{50}values for **39** and **40** respectively, on each cell line were ~ 4 µM, ~ 36 µM for HCE6 cells; ~ 14 µM, ~ 52 µM for Molt 4 cells; ~ 7 µM,~ 27 µM for HL60 line; and, ~ 10 µM, ~ 63 µM for K562 cells. A tree from the Amazon rain forest of Peru, Colombia and Brazil which has been used in the traditional medicine for treatment of tumors, is *H. succuba* (Apocynaceae, common name: "bellaco-caspi"). The first report about scientific validation for the use of this plant to treat the cancer was published by Perdue and Blomster 1978. The authors isolated to fulvoplumierin (**41**) from the plant stem bark after testing a 50% ethanol extract against KB cell line. While Castillo et al. (2007) studied the cytotoxicity on 3T3 line (using the SRB method) of plumericin (**42**) and isoplumericin (**43**) isolated from the stem bark. The IC_{50} values determined for **42** and **43** correspondingly, were 2 µM and 1 µM on cell line. An African plant is *K. africana* (syn. *K. pinnata*, Bignoniaceae), whose bark/fruit have been traditionally used as treatment of neoplastic diseases. Momekova et al. (2012) assessed the antineoplastic activity on two cell lines, and LLC bearing BDF1 mice from methanol extract of the plant stem bark. The IC_{50} values were 10 ± 3 µg/mL (LLC bearing-mice), 12 ± 4 µg/mL (MCF7 cells) and 15 ± 3 µg/mL (SKW3 line). Another important plant in the traditional Chinese medicine is *M. tenacissima* (Asclepiadaceae), which has been widely used for the treatment of cancers; and, more than decade ago, its application was approved to treat esophageal, gastric, and lung cancers, and hepatocell carcinoma by China Food and Drug Administration Office. Some authors have carried out the scientific validations against different cell lines. Hu et al. (2008) determined that 20 µg/mL of two compounds [tenacissimoside A (**44**, eq. 25 µM) and 11α-O-benzoyl-12β-O-acetyltenacigenin B (**45**, eq. 39 µM)] isolated from the plant increased the sensitivity of antitumor drugs, e.g., doxorubicin (18-fold and 16-fold), vinblastine (10-fold and 53-fold), puromycin (11-fold and 16-fold) and paclitaxel (6-fold and 326-fold) by HepG2/Dox cells. Additionally, other authors like Han et al. (2012, 2014, 2015) studied the synergistic effect between an extract of plant stem with other drug (e.g., gefitinib) against four non-small cell lung cancer lines. They found that the mixture (extract + gefitinib) as a therapeutical prepared resulted in a promising therapy to improve the effectiveness of gefitinib in resistant NSCLC. A species from Panaman flora is *M. blepharodes* (Celastraceae), which was studied for its ethnobotanical uses for the treatment of cancer by Rodriguez et al. (2005). These authors isolated three phenolic triterpenes [7-oxo-blepharodol (**46**), blepharotriol (**47**),

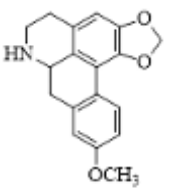
and 6-deoxoblepharodol (**48**)] from the plant bark and assessed the cytotoxicity (by MTT assay) on HeLa cell line. The IC_{50} values were ~ 12 µg/mL for **47**, ~ 14 µg/mL for **48** and ~ 20 µg/mL for **46**.

Ling et al. (1981) reported the potent cytotoxic effect of the ethanol extract from *M. emarginata* (Celastraceae) against P388 cell line. Kuo et al. (1989) isolated to emarginatine A (**49**), whose ED_{50} value was of 4 µg/mL on KB cells. Again, Kuo et al. (1990) established the ED_{50} values on KB cell line for chloroform, chloroform:methanol and ethyl acetate:acetone fractions along with emarginatine B (**50**) isolated from the plant stem, which were respectively, 0.4 µg/mL, 0.08 µg/mL, 0.04 µg/mL, and 0.4 µg/mL. While Kuo et al. (1994a) reported the isolation and the cytotoxicity against KB cell line of emarginatine E (**51**) and emarginatinine (**52**); the ED_{50} values were 2.5 µg/mL and 2.1 µg/mL, in that order. In the same year, Kuo et al. (1994b) isolated from the plant stem emarginatine F (**53**), which was cytotoxic against six cell lines. The ED_{50} values for **53** were < 0.1 µg/mL for RPMI7951 cells, 0.2 µg/mL for TE671 line, 0.5 µg/mL for KB cells, 0.7 µg/mL for P388 line, ~ 1 µg/mL for HCT8 cells and ~ 6 µg/mL for A549 line.

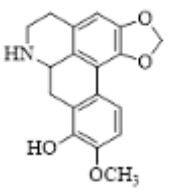
A perennial tree (endemic of India, and broadly distributed in Asia, Africa and other continents) important for the world is *M. azadirachta* (Meliaceae, syn. *Azadirachta indica*, common name: Neem), due to its extraordinary benefits (e.g., for ethnomedicine, agriculture, and domestic uses), and by its proximity with the cultures and civilization. In Ayurverdic medicine the leaves, bark, fruits, and seed-oil are widely used for treatment of cancers (Paul et al. 2011). Pan et al. (2014) estimated the cytotoxic effects against four cell lines of defatted more-polar fraction, and isolated compounds (type limonoids) from plant fruits by means of the MTT assay. The authors found that the fraction was active on all cell lines with IC_{50} values of ~ 3 ± 2 µg/mL – 21.9 ± 0.6 µg/mL. From this fraction, they isolated 20 compounds [limonoids and triterpenoid (e.g., some as **54**–**61**)], which showed some activity against the cancer lines. Thus, 19 compounds were effective on HL60 cells; 14 compounds were active against AZ521 line; nine compounds inhibited the SKBR3 cell growth; and, three compounds inhibited to A549 cell line. While two compounds were able to inhibit the four types of cancer cells: **56** and **60** (IC_{50} 5.0 ± 0.9 µg/mL – 82.3 ± 0.3 µg/mL); whereas four compounds were effective on three cell lines, i.e., **54**, **57** and **61** (IC_{50} 4.6 ± 0.6 µg/mL – ~ 54 ± 4 µg/mL), which were inactive on A549 cells, and **55** (IC_{50} 9.9 ± 0.6 µg/mL – ~ 94 ± 1 µg/mL), that was ineffective on AZ521 line. One compound (**58**) only was active against HL60 cells, with IC_{50} value of 4.9 ± 0.5 µg/mL. The remaining compounds (eight) inhibited two (HL60 and AZ521) of the four cell lines; the IC_{50} values were ranged among 2.8 ± 0.6 µg/mL – ~ 83 ± 1 µg/mL. As endpoint, the most active compounds against the cell lines were **59** (IC_{50} 2.8 ± 0.6 µg/mL, on HL60 cells; IC_{50} 3.2 ± 0.6 µg/mL, on AZ521 line), **56** (IC_{50} ~ 15 ± 2 µg/mL, on SKBR3 cells) and **60** (IC_{50} ~ 26 ± 2 µg/mL, on A549 line).

Takagi et al. (2014) also evaluated the cytotoxicity on five cell lines, of some isolated compounds (type limonoids) from the ethyl acetate fraction from neem leaves. The authors isolated 17 limonoid compounds, of which 15 compounds were active against the cell lines under study. Thus, 15 compounds were active on HL60 cells; 12 limonoids were effective against AZ521 line; 10 compounds inhibited the SKBR3 cell growth; and, seven limonoids inhibited to A549 cell line. On the other hand, six limonoid compounds inhibited the four types of cancer lines: **62**–**64**, **66**, **70** and **72** (IC_{50} 1.7 ± 0.1 µg/mL – ~ 98 ± 8 µg/mL); whereas four compounds were effective on three cell lines, i.e., **65**, **68**, **71** and **73** (IC_{50} ~ 9 ± 1 µg/mL – ~ 92 ± 4 µg/mL), these same compounds were ineffective on A549 cells. Only two compounds were effective on HL60 cells; and, two limonoids (**67** and **69**) were able to inhibit two (HL60 and AZ521) of the four cell lines; the IC_{50} values were ranged among 0.10 ± 0.01 µg/mL – ~ 76 ± 4 µg/mL. The most active compounds against the cell lines were **69** (*nimbolide*, IC_{50} 0.10 ± 0.01 µg/mL, on HL60 cells; IC_{50} 0.80 ± 0.01 µg/mL, on AZ521 line), **70** (IC_{50} ~ 1.7 ± 0.1 µg/mL, on SKBR3 cells) and **63** (IC_{50} ~ 8 ± 3 µg/mL, on A549 line).

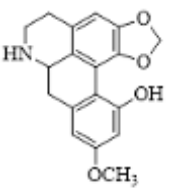
Other researchers have also verified the anticancer efficacy of different extracts from neem parts. Wu et al. (2014) evaluated the cytotoxicity on PC3 cell line of the methanol fractions (11) from the supercritical extract of neem leaves. Fractions 2, 3 and 5 resulted highly effective against PC3 line with IC_{50} values < ~ 2 µg/mL; whilst fractions 6–11 presented IC_{50} values between ~ 8 µg/mL and ~ 15 µg/mL. One of first reports about the isolation of compounds with cytotoxic properties from neem was published by Cohen et al. (1996). These authors measured the cytotoxic potential of six limonoids (included **69**) isolated of the seeds against N1E115, 143B.TK$^-$ and RAW264.7 cell lines. The IC_{50} values established were ~ 4 ± 1 µM (143B.TK$^-$ cells) and ~ 5 ± 1 µM (N1E115 and RAW264 lines) for *nimbolide*. Priyadarsini et al. (2010)



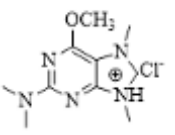
36



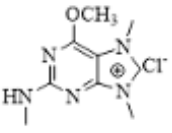
37



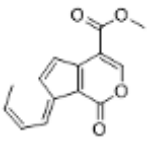
38



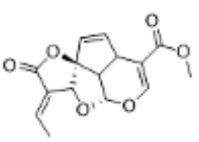
39



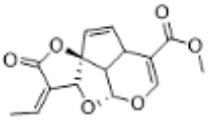
40



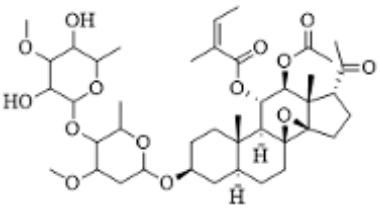
41



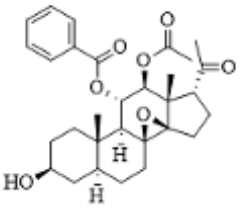
42



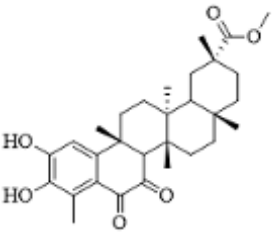
43



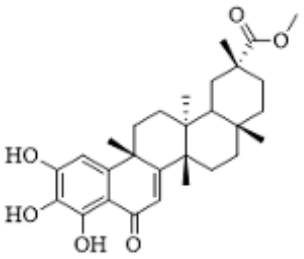
44



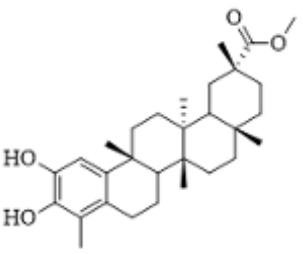
45



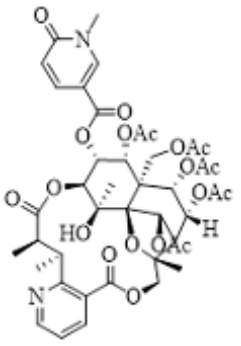
46



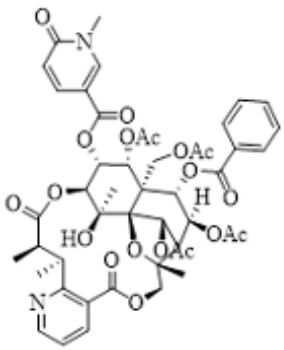
47



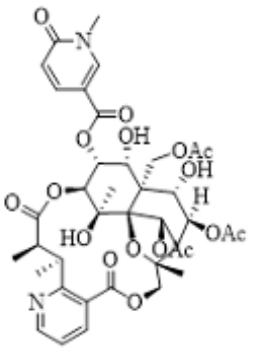
48



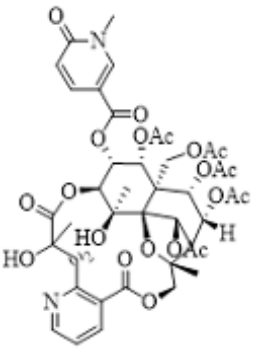
49



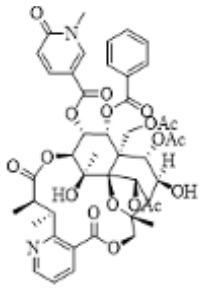
50



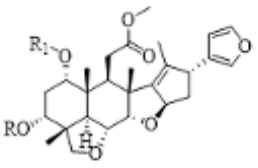
51



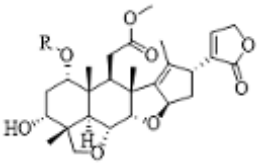
52



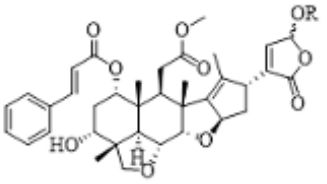
53



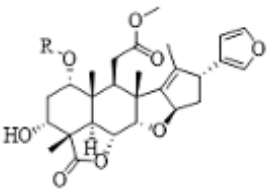
54 R: H-; R_1: CH_3-CH=C(-CH_3)-CO-
55 R: CH_3-CO-; R_1: C_6H_5-CH=CH-CO-
56 R: H-; R_1: C_6H_5-CO-



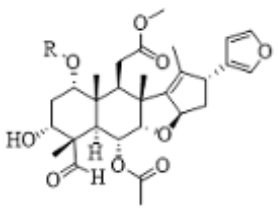
57 R: C_6H_5-CO-



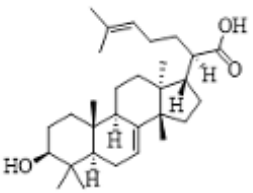
58 R: CH_2=C(-CH_3)-CO- (stereois. A)



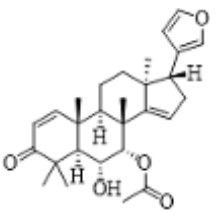
59 R: CH_2=C(-CH_3)-CO-



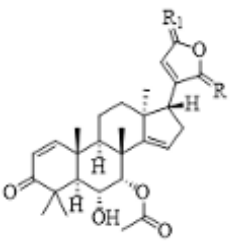
60 R: H-



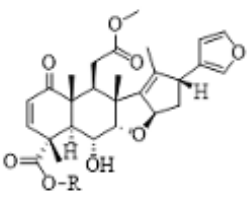
61



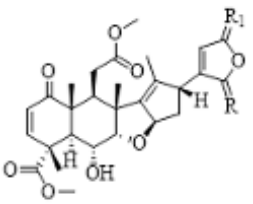
62



63 R: H-,OH-; R_1: O
64 R: O; R_1: H_2



65 R: CH_3
66 R: CH_3CH_2



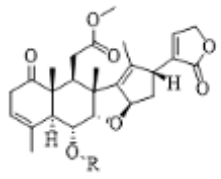
67 R: O; R_1: H-, OH-

studied the cytotoxic effect of nimbolide **69** on HeLa cancer line; 5 μM was the IC_{50} value. Other authors estimated the anticancer/antiproliferative properties of **69** isolated from *M. azadirachta*. Harish Kumar et al. (2009) found that IC_{50} value of **69** against BeWo cells was of ~ 1 μM (at 24 h). Roy et al. (2007) established that nimbolide (0.5–5.0 μM), isolated from neem flowers, showed antiproliferative activity against four cancer cell lines. The IC_{50} values were 1.1 μM (HL60 cells), 1.2 μM (U937 line), 1.4 μM (THP1 cells) and 1.7 μM (B16 line). Meanwhile, Elumalai et al. (2012) proved the inhibitory effects of nimbolide (IC_{50}: ~ 3 μM, at 48 h) on the invasive ability of MCF7 and MDAMB231 tumor cell lines. Babykutty et al. (2012) demonstrated that **69** (IC_{50}: ~ 2.6 μM, at 48 hours) inhibited the proliferation of the WiDr cancer cells by delaying its migration, invasive capacity, and angiogenesis, and inducing them apoptosis.

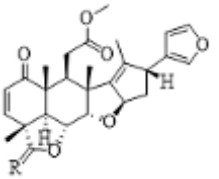
N. domestica (Berberidaceae) is the only species of *Nandina* found in China, and there, different parts of the plant (e.g., fruits, stems, roots and leaves) have been used in the indigenous medicine system

for the treatment of pharyngeal tumors and uterine bleeding. Peng et al. (2014) isolated 11 alkaloids from the plant fruit and determined the cytotoxic activity by the MTT test of a new compound (nandsterine **74**) against HL60 line. The IC_{50} value was ~ 52 μM. A millenary (ca. 5000 years) plant of Korean origin is *P. ginseng* (Araliaceae, common name: Ginseng), which has been used as prophylactic/curative agent for treatment different diseases, including cancer. The most used part is the root containing at least 28 active ginsenosides. Wang et al. (2007) determined the cytotoxicities (by MTT assay) on 12 cell lines of 11 saponins isolated from plant fruits. Three saponins (**75–77**) were the most active compounds against all cell lines tested, with IC_{50} values ranged between ~ 12 μM – ~ 69 μM for **76**, ~ 20 μM – ~ 72 μM for **75** and ~ 20 μM – ~ 78 μM for **77**. An herbaceous plant of great importance in the Mexican and Latin American traditional herbalist is *P. alliacea* (Phytolaccaceae, common name: anamú), which has been used to treat cancer. Rossi et al. (1990, 1993) and Jovicevic et al. (1993) determined the antiproliferative activity by the MTT test of an aqueous decoction from Anamú leaves against IM9 (human myeloma), Daudi (Burkitt's lymphoma) and Molt4 cell lines. They found that the decoction produced the cell growth inhibitions (%) of 80–90% on IM9 cells, 50–60% on Daudi and Molt4 lines. The IC_{50} value was 10 μg/mL on IM9 line. A Brazilian plant is *P. paniculata* (Amaranthaceae, called Brazilian ginseng) whose roots have been used in folk medicine as cancer therapy. Takemoto et al. (1983) isolated to pfaffic acid (**78**) from plant roots which showed high inhibitory effects against B16, HeLa (S3) and LLC cell lines at concentrations of 4–6 μg/mL. *P. peruviana* (Solanaceae) is native to western South America (Andes region, mainly Peru), and its fruits, in particular have been used in ethnomedicine for treating diseases such as cancer. Demir et al. (2014) evaluated the cytotoxic capacity of the ethanol extract from the plant fruit against Saos2 and Hep3B cell lines using the MTT method. The IC_{50} values found were ~ 15 μg/mL on Saos2 cells, and ~ 25 μg/mL on Hep3B. Yen et al. (2010) isolated to 4β-hydroxywithanolide E (**79**) from plant leaves and stem which showed anticancer property on H1299 cell line. The IC_{50} values were ~ 0.6 μg/mL and ~ 0.7 μg/mL at 24 hours and 48 hours, respectively.

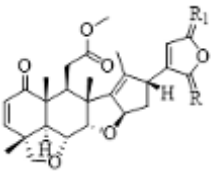
R. korthalsii (Araceae) is a native species from Borneo. Wong and Tan (1996a) examined the cytotoxicity (by the MTT assay) of petroleum ether extract from plant leaves against P388, Molt4, KB and SW620 cell lines. The ED_{50} values calculated were 8 μg/mL on KB cells, 12 μg/mL on P388 line, 13 μg/mL on SW620 cells and 14 μg/mL on Molt4 line. *R. javanica* (syn: *Brucea javanica*, Simaroubaceae) is a perennial shrub distributed mainly in Southeast Asia. In China, its fruits are used to treat cancer. Lau et al. (2008) confirmed that the ethanol extract from *Fructus Bruceae* (dried ripe fruits of *B. javanica*) presented cytotoxic activities against three cell lines. The IC_{50} values were 1.5 μg/mL, 2.5 μg/mL and 5.1 μg/mL for Capan1, Panc1 and SW1990 cancer cells, respectively. The tree *R. verniciflua* (Anacardiaceae, syn: *Toxicodendron vernicifluum*, common name: lacquer tree) is widely distributed in China, Korea and the Indian subcontinent. This plant has been used (although restricted) in traditional medicine to treat cancers for centuries. Kim et al. (2002) determined the cytotoxicity on four cancer cells of the hexane fraction (free of urushiols) from the plant bark. The GI_{50} values were ~ 13 μg/mL on Molt4F cells, ~ 16 μg/mL on SW620 line, ~ 19 μg/mL on ACHN cells and ~ 20 μg/mL on A549 line. Kim et al. (2015) estimated the cytotoxic potential of nine isolated compounds of two active fractions obtained from the ethanol extract (free of urushiols) of the plant bark against A549, SKOV3, SKMEL2 and HCT15 cell lines using SRB assay. Six compounds of nine resulted active against the four cell lines tested. The IC_{50} values for **80**–**85** on the four cell lines were between ~ 5 ± 1 μM – ~ 29 ± 1 μM. The most active compound with the lowest IC_{50} values (~ 5 ± 1 μM – ~ 10 ± 1 μM) was butein (**84**). Choi et al. (2016) investigated the effect of **84** on cell death of SKOV3/PAX line. They found that 10 μg/mL of compound inhibited the SKOV3/PAX cell growth (~ 50% population). Hong et al. (1999) isolated four urushiols (**86**–**89**) from the plant sap and screened the cytotoxic potential against 29 cell lines [PC3; OVCAR4, SKOV3, Molt4F, K562, RPMI8226, MDAMB235, MCF7/ADR, MCF7; SKMEL2, M14, LOXIMVI, UACC62, SNB75, SNB19, SF539; SW620, KM12, HCT116, HCT15, and Colo205, UO31,CAKI1, ACHN, NCIH522, NCtH23, NCIH226 and A549]. The GI_{50} values for **86** were ranged among ~ 0.6–8.6 μg/mL on 29 cancer cells; for **87** were between ~ 0.4–4.8 μg/mL on 28 cancer cells; for **88** were amongst ~ < 0.1–8.2 μg/mL on 28 cancer cells; and, for **89** were between ~ 0.2–5.4 μg/mL on 27 cancer cells. The most susceptible cell lines to the four urushiols were NCIH522, Molt4F and RPMI8226, with GI_{50} values < 1 μg/mL. Other cancer cells also sensitive to one of the urushiols were SKMEL2 for **86** (GI_{50}: 1 μg/mL), OVCAR4 for **87** (GI_{50}: 0.8 μg/mL) and M14 and SKMEL2 for **88** (GI_{50}: 0.2 μg/mL and 1 μg/mL).



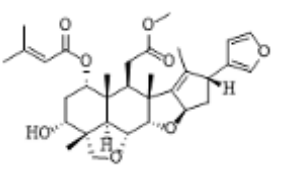
68 R: H



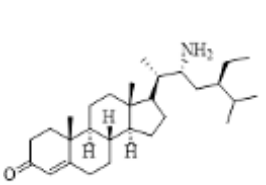
69 R: O
70 R: H_2



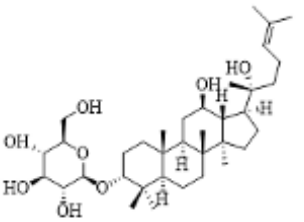
71 R: O; R_1: H_2
72 R: H-,OH-; R_1: O



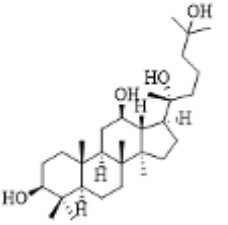
73



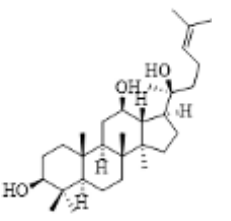
74



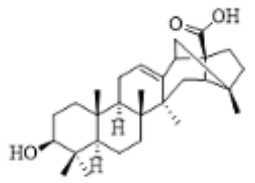
75



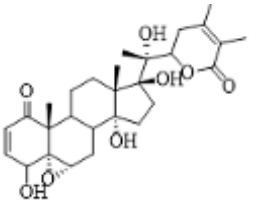
76



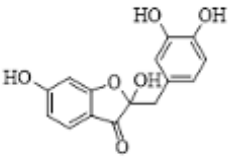
77



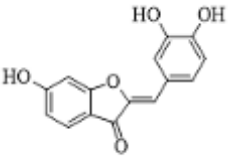
78



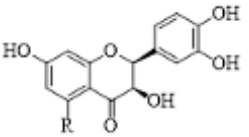
79



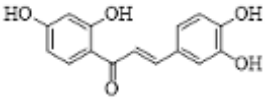
80



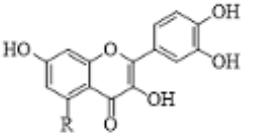
81



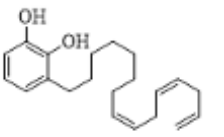
82 R: H
83 R: OH



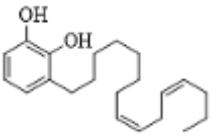
84



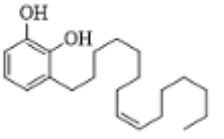
85



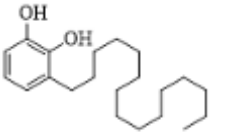
86



87



88



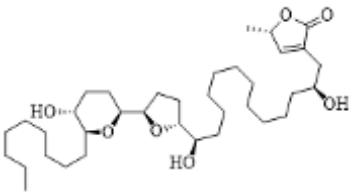
89

Another interesting species is *R. mucosa* (Annonaceae, common name: anonillo or cherimoya), which is a fruit tree distributed in Central America and West Indies, and in indigenous medicine its different parts have been used for treatment of cancer. Cytotoxic screening on brine shrimp and six cancer lines of the chloroform:methanol extract, methanol fraction and isolated jimenezin (**90**) from fruit seeds were carried out by Chávez et al. (1998) and Mata et al. (2001). They found that the LC_{50} (*A. salina*) values for the extract and fraction were ~ $4x10^{-1}$ µg/mL and 6 x 10^{-2} µg/mL, respectively. Similarly, the LC_{50} values of extract were < 1 x 10^{-3} µg/mL on A498 and PC3 lines, ~ 1.7 µg/mL on HT29 cells and ~ 7.0 µg/mL on PaCa2 line. Again, Chávez et al. (1999) isolated to membranacin (**91**) and desacetyluvaricin (**92**) from plant seeds, which were also highly active. The cytotoxic evaluation established that **90** was a

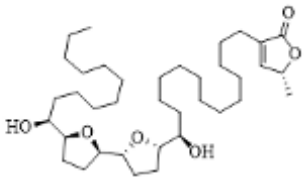
powerful cytotoxic agent on both brine shrimp and five cell lines (PaCa2, PC3, HT29, A549 and A498) tested, with LC_{50} values of ~ 6 x 10^{-3} µg/mL on *A. salina*, and ~ 2 x 10^{-4} µg/mL – ~ 5 x 10^{-2} µg/mL for the lines; whereas, **91** and **92** were strong cytotoxic agents against all systems tried: **92** and **91** obtained LC_{50} values of ~ 2 x 10^{-2} µg/mL and ~ 5 x 10^{-2} µg/mL on *A. salina*, respectively; and ED_{50} values of < 1 x 10^{-3} µg/mL – ~ 3 µg/mL. Shi et al. (1996, 1997) studied the cytotoxicity on six cell lines and brine shrimp of rollitacin (**93**), rollinacin (**94**), and rollinecins A (**95**) and B (**96**) isolated from plant leaves. They found that the four compounds were highly effective against the model systems evaluated; LC_{50} values presented by **93**–**96** were between ~ 1 x 10^{-1} µg/mL – ~ 4 µg/mL on *A. salina*, and ED_{50} values were among ~ 4 x 10^{-5} µg/mL – ~ 3 µg/mL on the cell lines. Gu et al. (1997) also determined the cytotoxicity against six cell lines of rodillecins C (**97**) and D (**98**) isolated from plant leaves. The authors verified that both compounds were effective on the six cell lines tested; **97** showed ED_{50} values of ~ 6 x 10^{-2} µg/mL – ~ 1 µg/mL; whilst, **98** had ED_{50} values of ~ 1 µg/mL – ~ 6 µg/mL.

S. glabra (Chloranthaceae, common name: Caoshanhu) is an evergreen shrub used in traditional Chinese medicine for treatment of cancers. Ni et al. (2013) screened the anticancer potential (on HL60 cell line) of 22 sesquiterpenoids isolated from the whole plant. The authors established that two compounds (**99** and **100**) of the eight new sesquiterpenoids were active, with IC_{50} values of 0.03 µM and 1.2 µM, respectively. Also, Wu et al. (2015) isolated 3,3'-biisofraxidin (**101**) from the plant. The IC_{50} value of **101** was ~ 20 µM on BGC823 cells. *S. baicalensis* (Lamiaceae, common name: Huang-Qin, Chinese skullcap) is a species native from East Asia and their roots have been used in traditional Chinese medicine for more than 2000 years as treatment of tumors. Kumagai et al. (2007) investigated the antitumor activities on 11 types of cancer cells of DMSO extract from the plant powder. They found that the ED_{50} values for Daudi and NCIH929 cancer cells were ~ 4.6 µg/mL and ~ 5 µg/mL, respectively. Gao et al. (2008) determined the cytotoxic effect of wogonin (**102**) against two cell lines. The IC_{50} values were ~ 11 ± 3 µg/mL (A549 line) and ~ 22 ± 3 µg/mL (SKLU1 cells). Ji et al. (2015) isolated 30 constituents [including **102**, baicalin (**103**) and baicalein (**104**)] from dried slices of the plant root and assessed the cytotoxic capacity against HepG2, SW480 and MCF7 cell lines by the MTS assay. They found that 10 µM **102**–**104** inhibited the cancer cell growth. Neves et al. (2011) evaluated the cytotoxicity against three cancer lines of **104**. The GI_{50} values were 7.7 ± 0.5 µM on A375-C5 cells, ~ 27 ± 3 µM on NCIH460 line and ~ 33 ± 2 µM on MCF7 cells. Huang et al. (2010) determined that 50 µM of **102** inhibited the HL60 cancer cell growth ~ 48%. *S. tamariscina* (Selaginellaceae) is another traditional Chinese herb used as therapy for some kinds of cancers. Li et al. (2014a) determined the anticancer properties of the ethyl acetate extract from the plant against three cell lines. The IC_{50} values were 3.2 ± 0.4 µg/mL on HT29 line, 3.2 ± 0.8 µg/mL on HeLa cells and ~ 8 ± 2 µg/mL on Bel7402 cells. *S. torvum* (Solanaceae, common name: turkey berry) is a shrub native to southern Mexico and Central America and is also distributed in Asia and Africa. In herbal medicine from Indonesia, it is used as an anticancer agent. Balachandran et al. (2015) investigated the anticancer activity of methyl caffeate (**105**) isolated from plant fruits against MCF7 cell line. The IC_{50} value was ~ 0.6 µM. Li et al. (2014b) isolated five new steroidal glycosides from plant fruits and evaluated the cytotoxicity against A375 cancer cell. The authors found that four of them were moderately active, with IC_{50} values ranged between ~ 40–260 µM.

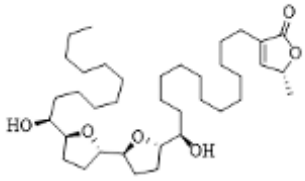
S. venosa (Menispermaceae) is a shrub native to the eastern and southern Asia and in traditional Thai medicine is used for treating cancer. Leewanich et al. (2011) investigated the antineoplastic effect on NCIH187 cancer line of ethanol extract from the plant. The IC_{50} value was ~ 5 µg/mL. Le et al. (2017) studied the anticancer properties against HeLa, MDAMB231 and MCF7 cell lines of dichloromethane/butanol fractions, and five isolated alkaloids from plant tubers. The authors found that all fractions were active on the three cell lines: the IC_{50} values were ~ 11 ± 3 µg/mL – 14.1 ± 0.6 µg/mL for dichloromethane fraction, and ~ 18 ± 3 µg/mL – ~ 26 ± 2 µg/mL for butanol fraction. Besides, four compounds (**106**–**109**) were efficacious against the cancer lines; the IC_{50} values were 3.3 ± 0.2 µM – 6.5 ± 0.4 µM for **107** (stephanine); ~ 11 ± 2 µM – ~ 19 ± 1 µM for **106**; ~ 30 ± 5 µM – ~ 48 ± 2 µM for **108**, and ~ 39 ± 6 µM – ~ 70 ± 11 µM for **109**. Nantapap et al. (2010) investigated the antiproliferative effects of four alkaloids isolated from plant tubers on K562, K562/Adr, GLC4 and GLC4/Adr cancer lines. They found that **108**–**110** were effective on cancer cells; the IC_{50} values were ~ 7 ± 2 µg/mL – ~ 10 ± 4 µg/mL for **108** (crebanine); ~ 9 ± 1 µg/mL – ~ 14 ± 4 µg/mL for **109** (o-methylbulbocapnine); and ~ 20 ± 5 µg/mL – ~ 61 ± 6 µg/mL for **110**. Niwat et al. (2001) assessed the cytotoxic activities on MCF7 line of the ethanol



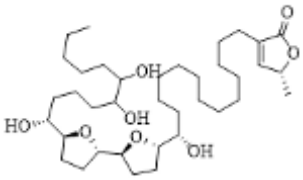
90



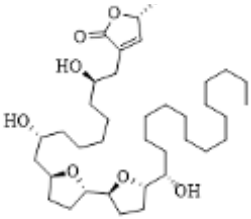
91



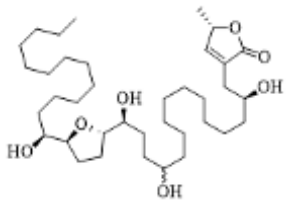
92



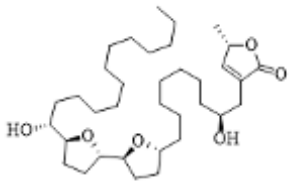
93



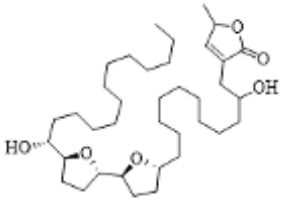
94



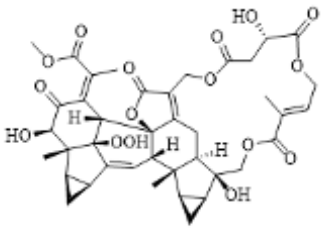
95,96



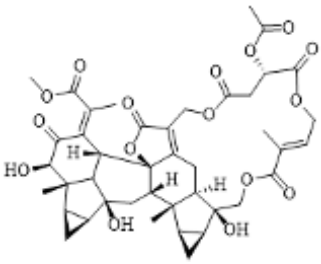
97



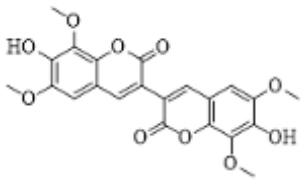
98



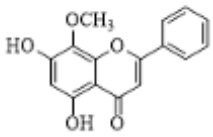
99



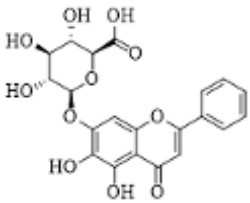
100



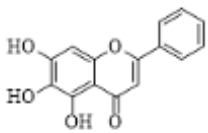
101



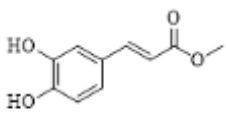
102



103



104



105

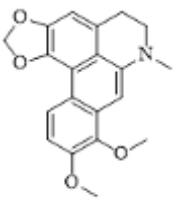
extract and two isolated alkaloids from plant tubers. The IC_{50} values were ~ 12 µg/mL (for extract) and ~ 5–6 µg/mL (for **111** and **108**).

T. avellanedae (Bignoniaceae) is a tree found in the northeast of Brazil. The purple bark (Taheebo) of this plant has been used by the Callawaya tribe for treatment of skin cancer. Yamashita et al. (2007, 2009)

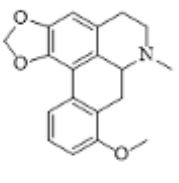
studied the cytotoxic effects of naphthoquinone (**112**), lapachol (**113**) and β-lapachone (**114**) against three cell lines. The EC_{50} values of **112** were ~ 0.1 μM, ~ 0.8–1 μM and ~ 0.5–4 μM on PC3, A549 and MCF7 cancer cells, respectively; the EC_{50} values of **113** and **114** on the same cell lines were ~ 1–2 μM, ~ 4–5 μM and ~ 10 μM. Zhang et al. (2015) isolated two furanonaphthoquinones from *T. avellanedae* inner bark, which showed antiproliferative effects on A549, SiHa and MCF7 cancer lines. The IC_{50} values were 0.50 ± 0.06 μM – 1.2 ± 0.3 μM for **112**; 1.1 ± 0.1 μM – 2.0 ± 0.4 μM for **115**; 2.0 ± 0.3 μM – 3.4 ± 0.6 μM for **116**; and 10.2 ± 0.4 μM – > ~ 14 μM for **117**. *T. rosea* (common name: pink trumpet tree) is a tree native from Mexico to Venezuela and Ecuador, used in traditional medicine for treating tumors. Sichaem et al. (2012) isolated 13 compounds from plant roots and studied the cytotoxic potential of the compounds against KB and HeLa cell lines. They found that 13 compunds were active on the two cancer lines; nonetheless, the most active were **112**, **118**–**121**. The IC_{50} values, respectively were ~ 0.5 μg/mL and ~ 0.8 μg/mL for **118**, ~ 1.8 μg/mL and ~ 0.7 μg/mL for **119**, ~ 1.4 μg/mL and ~ 1.2 μg/mL for **112**, ~ 2.0 μg/mL and ~ 4.2 μg/mL for **120**, and ~ 16 μg/mL and ~ 14 μg/mL for **121**.

T. wilfordii (Celastraceae, common name: thunder god vine) is a shrub native to Southeast Asia, used in traditional Chinese medicine from ancient times for the treatment of different ailments including cancer. Gao et al. (2017) isolated 13 compounds from plant dried roots and screened the cytotoxic effects on four cell lines. The authors found that only five compounds were effective on the cancer cells with IC_{50} values between ~ 11 ± 2 μM – ~ 88 ± 1 μM. The most active compounds were **122** and **123** on HepG2 cells with IC_{50} values of ~ 11 ± 2 μM and ~ 18 ± 2 μM, along with **124** on A549 line with IC_{50} value of ~ 16 ± 2 μM. Gao et al. (2016) isolated 21 diterpenoids from plant dried roots and evaluated the cytotoxic potential against six cancer lines. The results revealed that **125** and **126** (triptotin A) were the most active compounds against all cells, with IC_{50} values between ~ 5 ± 2 μM – ~ 23 ± 2 μM. The lowest IC_{50} values were ~ 5 ± 2 μM and ~ 6 ± 2 μM for **125** on HepG2 and MCF7 lines, ~ 8 ± 2 μM for **125** and **126** on Bcap37 cells, ~ 9 ± 2 μM for **125** on U251 line, ~ 11 ± 3 μM for **126** and **127** on A549 cells, and ~ 17 ± 3 μM for **128** on Hep3B line. Fan et al. (2016) isolated 17 compounds from plant dried stems and determined the cytotoxicity on A549 and A549T cancer cells. The authors found that five of the 17 compounds were active on the A549T line with IC_{50} values among ~ 11 ± 2 μM – 54.4 ± 0.3 μM; while, only triptofordin B (**129**) was effective against A549 cancer cells. The most active compound on A549T and A549 cell lines was **129** with IC_{50} values of ~ 11 ± 2 μM and ~ 21 ± 2 μM, respectively. Yang et al. (2011) isolated eight triterpenoids from plant roots and estimated the cytotoxic effect against HeLa cancer cells. The most active compounds were tripterfrielanons A (**130**) and B (**131**) with IC_{50} values of 8.5 μg/mL and 25 μg/mL, individually.

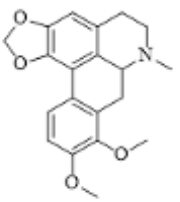
V. album (Santalaceae, common name: mistletoe) is a perennial hemiparasite shrub native to Europe and Asia, which has been used in folk medicine for both treatment and in complementary therapies against cancer. Weissenstein et al. (2016) investigated the effect of a standardized preparation on the action of Trastuzumab (drug used against breast cancer). The authors also determined the cytotoxic effect of standardized extract on SKBR3 cancer cells. They found that 1 μg/mL or ≥ 10 μg/mL of extract produced a cell growth inhibition > 60% or ~ 100%, respectively. Finally, *W. somnifera* (Solanaceae, common name: Ashwagandha, Indian ginseng) is a perennial herb native from the dry regions of Asia and other continents such as Africa. It is an important plant in Ayurvedic and traditional Chinese medicines due to its wide uses for treatment of several diseases, including cancer (Rai et al. 2016). Nema et al. (2013) screened the cytotoxicity of hydroalcohol extract from plant leaves against three cancer lines. The IC_{50} values were ~ 10 ± 1 μg/mL (MCF7 cells), ~ 11 ± 1 μg/mL (A459 line) and ~ 13 ± 1 μg/mL (PA1 cells). Jayaprakasam et al. (2003) isolated 12 whitanolides from plant dried leaves and evaluated the cytotoxic potential on four types of cancer cells. The results showed that 10 whitanolides exhibited cytotoxicity on all cancer lines tested with IC_{50} values between 0.24 ± 0.01 μg/mL – ~ 24 ± 1 μg/mL. **132**–**134** (3-dihydrowithaferin A) were the most active compounds with IC_{50} values among 0.24 ± 0.01 μg/mL – 0.9 ± 0.2 μg/mL. The lowest IC_{50} values were 0.24 ± 0.01 μg/mL for **132** (whitaferin A) on NCIH460 line; 0.36 ± 0.04 μg/mL, and 0.5 ± 0.2 μg/mL for **132** and **133** (viscosalactone B) on HCT116 cells; 0.28 ± 0.04 μg/mL for **132** on SF268 line; and 0.27 ± 0.04 μg/mL for **132** on MCF7 cells. Choudhary et al. (2015) isolated two whitanolides (included **132**) from methanol extract of plant aerial parts and evaluated the cytotoxicity on NCIH460 line for both the extract and whitanolides. The extract showed GI_{50} and LC_{50} values of 1.5 ± 0.2 μg/mL and



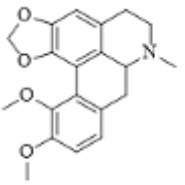
106



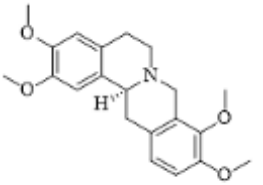
107



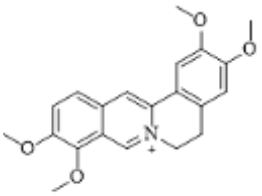
108



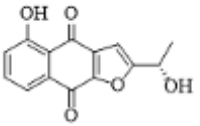
109



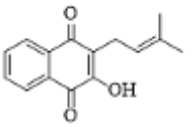
110



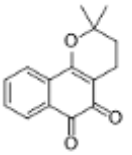
111



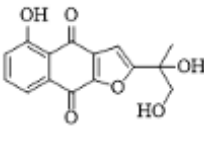
112



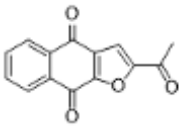
113



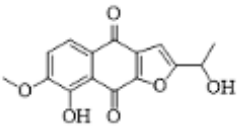
114



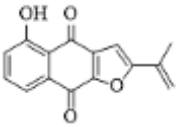
115



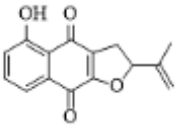
116



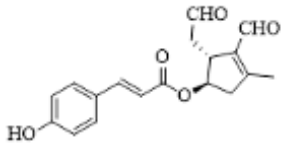
117



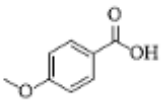
118



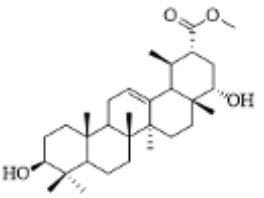
119



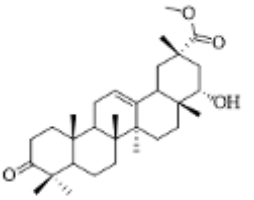
120



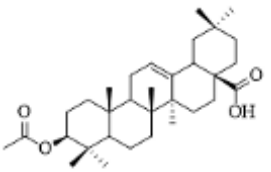
121



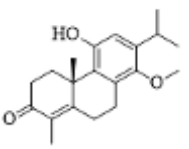
122



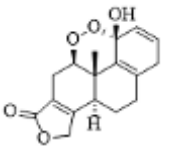
123



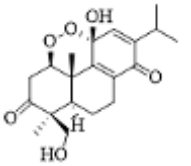
124



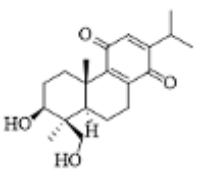
125



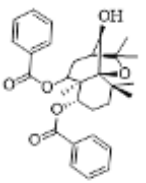
126



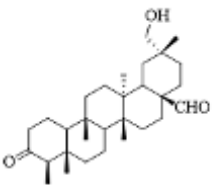
127



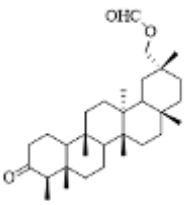
128



129

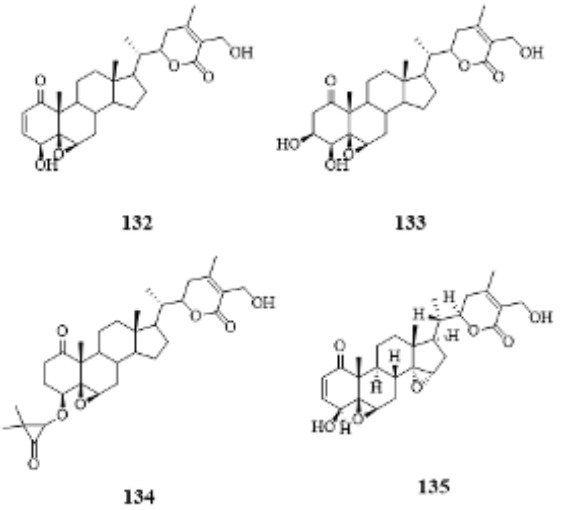


130



131

7.60 ± 0.05 μg/mL; whilst **132** presented GI_{50} and LC_{50} values of 0.18 ± 0.01 μg/mL and 0.45 ± 0.01 μg/mL; and, **135** exhibited GI_{50} and LC_{50} values of 1.5 ± 0.1 μg/mL and 8.5 ± 0.2 μg/mL.



Development of anticancer/antitumor drugs from chemical constituents of plants in accordance with patent analysis

In order to assess the innovation/inventive activities related to medicinal plants with anticancer/antitumor properties along with development of anticancer/antitumor drugs, the Derwent Innovations Index (Clarivate Analytics 2017) database was used. Five hundred and ten records of patents were obtained, which were analyzed by means of VantagePoint software (VP student, Search Technology). Figure 1.4 contains the inventive dynamic on 157 medicinal plants and antitumor/anticancer properties, in the last 20 years. An irregular behavior of the patent numbers per year could be observed in the timeline 1997–2006. Since 2007 an increase on the number of patents was evidenced: 2015 and 2016 were the most active years with 99 patents and 89 patents, respectively. To date (2017), only 44 records have been found. Considering the distribution of patents per offices (top five), it was found that China reported 338 patents, followed by South Korea (66 patents), United States (47 patents), Switzerland (10 patents), and Japan (nine patents). The plants with the highest number of patents related to anticancer/antitumor properties were: *S. lyratum* (56 patents), *C. longa* (54 patents), *M. tenacissima* (54 patents), *S. glabra* (44 patents), *C. roseus* (30 patents) and *W. somnifera* (14 patents). In agreement with the inventions correlated to applications on a specific cancer of an isolated compound/extract of plants, the following assignments were found: patents attendant to lung cancer (65 records), patents associated to gastric cancer (57 records), patents related to breast cancer (49 records), patents correspondent to liver cancer (42 records), patents mentioning effects on colorectal cancer (24 records), patents stating results on cervical cancer (22 records), etc. The patents were found from academic institutions, private corporations and natural persons, mainly: e.g., Qingdao Tumour Hospital (89 patents), Yinchuan Shanghetu New Technology Dev Co. (20 patents), Jinan Xinshidai Medical Technology Co. (eight patents), Fenghua Kechuang Technology Service Co. (four patents), Lifeline Nutraceuticals Corp. (four patents).

With the purpose of identifying what the plants validated on the different types of cancer and protected by patents, a correlational matrix was elaborated (Fig. 1.5). The analysis of the figure showed that *S. lyratum* and *C. longa* were the most protected plants, which had the greatest application/evaluation on the different types of cancer (21); followed by *M. tenacissima* (20 types of cancer) and *S. glabra* (18 types of cancer). Nonetheless, individual plants had a highest patent numbers depending on type of cancer, f.i., *S. lyratum* related to gastric cancer (14 registers), and *C. longa* associated to breast cancer (9 records). On the other hand, the main type of cancer on which the highest medicinal plant numbers (15) have been protected, was lung cancer; followed by, breast cancer (13 plants), and liver cancer (10 plants).

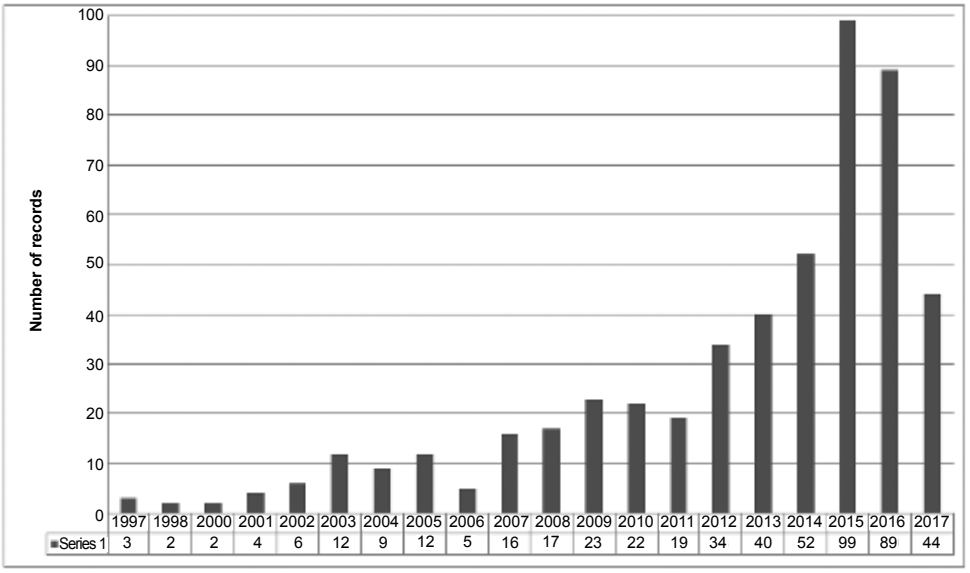


Fig. 1.4

Source: Bibliometry Unit – CRAI-Library, Universidad Santo Tomás (Bucaramanga). Calculations based on Derwent Innovations Index (Clarivate, Analytics, 2017) database, processed with VantagePoint software (VP Student, Search Technology).

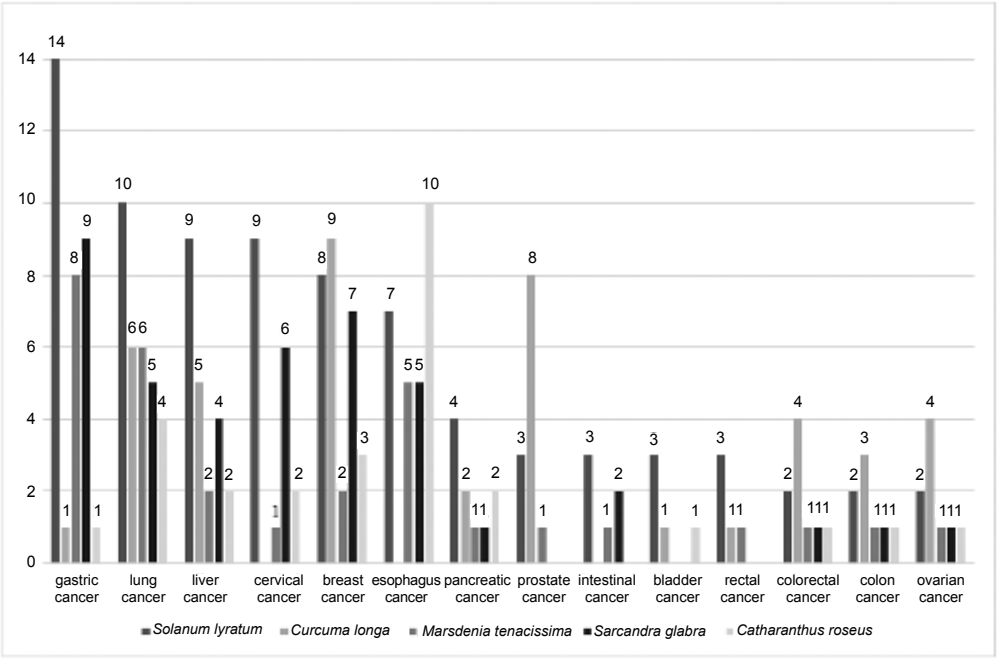


Fig. 1.5

Source: Bibliometry Unit – CRAI-Library, Universidad Santo Tomás (Bucaramanga). Calculations based on Derwent Innovations Index (Clarivate Analytics, 2017), processed with VantagePoint software (*VP Student, Search Technology*).

Table 1.2 includes some patents conferred from the medicinal plants (isolated compounds/extracts) with anticancer/antitumor properties scientific validated. This table contains the names of medicinal plant, patent codes, patent titles, IPC classifications, assignees, and dates.

Table 1.2. Some patent applications from the medicinal plants (isolated compounds/extracts) with anticancer/antitumor properties scientifically validated.

Medicinal plant	Patent code	Title	IPC classifications	Asignees	Date
Annona squamosa	CN 101401806 A CN 101342202 A	Use of *A. squamosa* bullatacin for preparing drug used to treat lung, breast or liver cancers	A61K-031/365, A61P-035/00	Univ. Nanjing Chinese Medicine & Pharmaco	Sep 17, 2008
	CN 1593495 A CN 1269521 C	Compound medicine for treating cancer, in which the medicament is prepared from total *A. squamosa*, *Polygoni multiflori*, *Curcuma aromatica*	A61K-035/78, A61K-009/08, A61P-035/00, A61K-036/13, A61K-036/22, A61K-036/53, A61K-036/704, A61K-036/75, A61K-036/9066	Xing Lok Road	Mar 16, 2005 Aug 16, 2006
	CN 1319424 A	Anticancer medicine of *A. squamose* extractum and preparation method thereof	A61K-035/78, A61K-009/06, A61P-035/00	Univ. Nangjing Trad. Chinese Medici	Oct 31, 2001
Artemisiae argyi	CN 101468184 B	Medicament for treating digestive system cancer and preparation method thereof	A61K35/24, A61K36/9066, A61K9/20, A61K9/48, A61P35/00	Haitang Liu	Sep 7, 2011
Curcuma longa	GB 2531850 B	Phyto-active based anti-cancer formulation	A61K36/324, A61K36/87, A61K36/906, A61P35/00	Omni Cure Ltd.	Jun 28, 2017
	CN 102302742 B	Traditional Chinese medicine composition for treating cardiac and esophageal cancers	A61K35/36, A61K35/64, A61K36/9066, A61P35/00	Hongkun Zhao	Oct 24, 2012
Mahonia fortunei	CN 102008685 B	Chinese medicinal powder for treating liver, lung and pancreatic cancers	A61K36/9066, A61K9/14, A61P1/16, A61P11/00, A61P35/00	Xuezhe Gao	Jul 4, 2012
Marsdenia tenacissima	CN 101215313 B	*M. tenacissima* carbon-21 steroid saponin mixture with antineoplastic effect	A61K31/7048, A61K36/185, A61P35/00, C07J71/00	Zhejiang Academy Medical Sci.	Jun 9, 2010
	CN 101463065 B	*M. tenacissima* extract	A61K31/7048, A61K36/27, A61P35/00, C07J71/00	Jiangsu Simcere Pharm. Res. Co.	Jul 4, 2012
Petiveria alliacea	WO 2011039629 A2 CA 2776446 A1 US 2012294897 A1 BR 112012009362 A2	Bioactive fraction used for preparing drug for treating cancer, is obtained from *P. alliacea* by bio-engineered, standardized and analytical processes…	A61K-000/00, A61K-036/185, A61P-035/00, A61K-031/7048, A61K-038/17, A61K-039/39, A61K-036/28	Univ. Javeriana Pontificia	Apr 07, 2011 Nov 22, 2012 May 27, 2014 Jun 07, 2016
Sarcandra glabra	CN 101919967 B	Traditional Chinese drug for treating esophagus cancer	A61K35/50, A61K35/62, A61K35/646, A61K36/898	Yi Junyu	Dec 7, 2011
	CN 101703726 B	Medicament for treating lung cancer	A61K35/57, A61K35/646, A61K36/9064, A61P35/00	Yuwei He	Sep 8, 2010
	CN 101972454 B	Medicament for treating lymphoma	A61K35/413, A61K35/57, A61K35/583, A61K36/9066	Yuwei He	Oct 5, 2011

Table 1.2 contd. ...

...Table 1.2 contd.

Medicinal plant	Patent code	Title	IPC classifications	Asignees	Date
Sarcandra glabra *Solanum lyratum*	CN 102133378 B	Medicament for treating breast cancer	A61K35/57, A61K35/586, A61K35/618, A61P35/00	Yuwei He	Feb 29, 2012
Solanum lyratum	CN 101049449 B	Composition of Chinese traditional medicine for malignant tumor	A61K35/32, A61K36/8994, A61P35/00	Shi-Jie Li	Feb 29, 2012
	CN 101940708 B	Traditional Chinese medicinal preparation for curing malignant pleural effusion	A61K36/8966, A61P35/00, A61P7/10	Feng Cao	Jun 6, 2012
Viscum album	GR 1008177 B	Method for the treatment of fir-based extract exhibiting biological action…	A61K36/15	Polychronis Argyrios Konstantinou	Apr 17, 2014
	MD 743 F1	Remedy for immunity system and hormonal homeostasis normalization	A61K35/64	Victor Babco; Petru Pihut; Vladislav Babco	Jun 30, 1997
	GB 1152618 A	Process for the preparation of a physiologically active protein material	A23J1/10, A61K36/00, A61K36/185, C07K14/415	Ciba Ltd.	May 21, 1969

Conclusion

The scientific validation of the ethnobotanical uses of medicinal plants for cancer treatment is a topic that has been strengthening since the last 10 years, based on the scientific dynamics observed in the analysis of text mining carried out; at present, there is a strong and persistent interest in the scientific community and the pharmaceutical industries for finding/obtaining new drugs with the greatest effectiveness/selectivity, least side effects and lowest cost, which can act by different mechanisms of action in order to: improve the quality of life of people with cancer, or find a definitive cure for the disease. Some of the most effective drugs commercially available for the treatment of cancer were isolated from nature (plants) and perhaps from the plants themselves, the best molecules could be obtained with the benefits mentioned above. ***As a final point, there are still medicinal plants used in ethnomedicine to treat cancer from which its extracts/fractions/isolated compounds have not yet been scientifically validated by in vitro and in vivo methods, or in other cell lines and animal models. In the same sense, preparations/isolated compounds from the plants which were evaluated on in vivo assays (animal models), not all they have been submitted to preclinical (phase 0) and clinical studies (phases I–IV), together with safety, efficacy, and quality assessments that are necessary for the development of commercially available drugs. Therefore, scientists who study medicinal plants (e.g., ethnobotanists, ethnopharmacologists, biologists, molecular biologists, chemists, pharmacologists, toxicologists, pharmacists, etc.) could still take part to fill each knowledge gap in this area and thus contribute to solving in part this problem/disease.***

Acknowledgement

The authors would like to thank: Universidad del Norte, Division of Basic Sciences; and, Universidad Santo Tomás de Aquino (Bucaramanga) by using of the Bibliometric Unit.

References

Ahmed, S., Rahman, A., Mathur, M., Athar, M. and Sultana, S. 2001. Anti-tumor promoting activity of *Asteracantha longifolia* against experimental hepatocarcinogenesis in rats. Food Chem. Toxicol. 39: 19–28.

Alaikov, T., Konstantinov, S.M., Tzanova, T., Dinev, K., Topashka-Ancheva, M. and Berger, M.R. 2007. Antineoplastic and anticlastogenic properties of curcumin. Ann. N.Y. Acad. Sci. 1095: 355–370.

Alonso-Castro, A.J., Villarreal, M.L., Salazar-Olivo, L.A., Gomez-Sanchez, M., Dominguez, F. and Garcia-Carranca, A. 2011. Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. J. Ethnopharmacol. 133: 945–972.

Arbiastutie, Y., Marsono, D., Hartati, M.S. and Purwanto, R. 2017. The potential of understorey plants from Gunung Gede Pangrango National Park (West Java, Indonesia) as cervixs anticancer agents. Biodiversitas J. Biol. Divers 18: 109–115.

Arkhipov, A., Sirdaarta, J., Rayan, P., McDonnell, P.A. and Cock, I.E. 2014. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacogn. Commun. Bangalore 4: 62–76.

Babykutty, S., Priya, P.S., Nandini, R.J., Suresh Kumar, M.A., Nair, M.S., Srinivas, P. and Gopala, S. 2012. Nimbolide retards tumor cell migration, invasion, and angiogenesis by downregulating MMP-2/9 expression via inhibiting ERK1/2 and reducing DNA-binding activity of NF-κB in colon cancer cells. Mol. Carcinog. 51: 475–490.

Balachandran, C., Emi, N., Arun, Y., Yamamoto, Y., Ahilan, B., Sangeetha, B., Duraipandiyan, V., Inaguma, Y., Okamoto, A., Ignacimuthu, S., Al-Dhabi, N.A. and Perumal, P.T. 2015. *In vitro* anticancer activity of methyl caffeate isolated from *Solanum torvum* Swartz. fruit. Chem. Biol. Interact. 242: 81–90.

Breza, T.S., Halprin, K.M. and Taylor, J.R. 1975. Photosensitivity reaction to vinblastine. Arch. Dermatol. 111: 1168–1170.

Castillo, D., Arevalo, J., Herrera, F., Ruiz, C., Rojas, R., Rengifo, E., Vaisberg, A., Lock, O., Lemesre, J.-L., Gornitzka, H. and Sauvain, M. 2007. Spirolactone iridoids might be responsible for the antileishmanial activity of a Peruvian traditional remedy made with *Himatanthus sucuuba* (Apocynaceae). J. Ethnopharmacol. 112: 410–414.

Cates, R.G., Prestwich, B., Innes, A., Rowe, M., Stanley, M., Williams, S., Thompson, R., McDonald, S., Cates, S., Shrestha, G., Soria, J.A.F., Espinoza, L.V., Ardon, C., Galvez, B., Diaz, M.R., Coronado, F.S., Garcia, J.R., Arbizu, D.A. and Martinez, J.V. 2013. Evaluation of the activity of Guatemalan medicinal plants against cancer cell lines and microbes. J. Med. Plants Res. 4: 2616–2627.

Chávez, D., Acevedo, L.A. and Mata, R. 1998. Jimenezin, a novel annonaceous acetogenin from the seeds of *Rollinia mucosa* containing adjacent tetrahydrofuran-tetrahydropyran ring systems. J. Nat. Prod. 61: 419–421.

Chávez, D., Acevedo, L.A. and Mata, R. 1999. Tryptamine derived amides and acetogenins from the seeds of *Rollinia mucosa*. J. Nat. Prod. 62: 1119–1122.

Chen, M., Ni, Y., Duan, H., Qiu, Y., Guo, C., Jiao, Y., Shi, H., Su, M. and Jia, W. 2008. Mass spectrometry-based metabolic profiling of rat urine associated with general toxicity induced by the multiglycoside of *Tripterygium wilfordii* Hook. f. Chem. Res. Toxicol. 21: 288–294.

Chen, Y., Chen, J.-W., Wang, Y., Xu, S.-S. and Li, X. 2012. Six cytotoxic annonaceous acetogenins from *Annona squamosa* seeds. Food Chem. 135: 960–966.

Cheng, A.-S., Chang, W.-C., Cheng, Y.-H., Chen, K.-Y., Chen, K.-H. and Chang, T.-L. 2012. The effects of davallic acid from *Davallia divaricata* Blume on apoptosis induction in A549 lung cancer cells. Molecules 17: 12938–12949.

Choi, H.S., Kim, M.K., Choi, Y.K., Shin, Y.C., Cho, S.-G. and Ko, S.-G. 2016. *Rhus verniciflua* Stokes (RVS) and butein induce apoptosis of paclitaxel-resistant SKOV-3/PAX ovarian cancer cells through inhibition of AKT phosphorylation. BMC Complement. Altern. Med. 16: 122 (7 p.).

Choi, S.E., Kim, K.H., Kwon, J.H., Kim, S.B., Kim, H.W. and Lee, M.W. 2008. Cytotoxic activities of diarylheptanoids from *Alnus japonica.* Arch. Pharm. Res. 31: 1287–1289.

Choudhary, B., Shetty, A. and Langade, D.G. 2015. Efficacy of Ashwagandha (*Withania somnifera* [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. Ayu 36: 63–68.

Coatti, G.C., Marcarini, J.C., Sartori, D., Fidelis, Q.C., Ferreira, D.T. and Mantovani, M.S. 2016. Cytotoxicity, genotoxicity and mechanism of action (via gene expression analysis) of the indole alkaloid aspidospermine (antiparasitic) extracted from *Aspidosperma polyneuron* in HepG2 cells. Cytotechnology 68: 1161–1170.

Cohen, E., Quistad, G.B., Jefferies, P.R. and Casida, J.E. 1996. Nimbolide is the principal cytotoxic component of neem-seed insecticide preparations. Pestic. Sci. 48: 135–140.

Coria-Téllez, A.V., Montalvo-Gónzalez, E., Yahia, E.M. and Obledo-Vázquez, E.N. 2017. *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab. J. Chem. (in press).

Damayanthi, Y. and Lown, J.W. 1998. Podophyllotoxins: current status and recent developments. Curr. Med. Chem. 5: 205–252.

De Melo, J.G., Santos, A.G., de Amorim, E.L.C., do Nascimento, S.C. and de Albuquerque, U.P. 2011. Medicinal plants used as antitumor agents in Brazil: an ethnobotanical approach. Evid. Based Complement. Alternat. Med. 2011: 365359.

De Solla Price, D.J. 1963. Little science, big science. Columbia Univ. Press, New York.

Demir, T., Ozen, M.O. and Hameş-Kocabaş, E.E. 2014. Antioxidant and cytotoxic activity of *Physalis peruviana*. Med. Plant Res. 4: 30–34.

Desai, A.G., Qazi, G.N., Ganju, R.K., El-Tamer, M., Singh, J., Saxena, A.K., Bedi, Y.S., Taneja, S.C. and Bhat, H.K. 2008. Medicinal plants and cancer chemoprevention. Curr. Drug Metab. 9: 581–591.

Elumalai, P., Gunadharini, D.N., Senthilkumar, K., Banudevi, S., Arunkumar, R., Benson, C.S., Sharmila, G. and Arunakaran, J. 2012. Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. Toxicol. Lett. 215: 131–142.

Fan, D., Zhu, G.-Y., Chen, M., Xie, L.-M., Jiang, Z.-H., Xu, L. and Bai, L.-P. 2016. Dihydro-β-agarofuran sesquiterpene polyesters isolated from the stems of *Tripterygium regelii*. Fitoterapia 112: 1–8.

FDA. 2017. FDA approved drugs in oncology. https://www.centerwatch.com/drug-information/fda-approved-drugs/therapeutic-area/12/oncology (accessed 8.11.17).

Gao, C., Wang, D., Zhang, Y., Huang, X.-X. and Song, S.-J. 2016. Kaurane and abietane diterpenoids from the roots of *Tripterygium wilfordii* and their cytotoxic evaluation. Bioorg. Med. Chem. Lett. 26: 2942–2946.

Gao, C., Lou, L.-L., Wang, D., Zhang, Y., Huang, X.-X. and Song, S.-J. 2017. Chemical constituents from the roots of *Tripterygium wilfordii* and their cytotoxic activity. J. Asian Nat. Prod. Res. 19: 725–731.

Gao, J., Sanchez-Medina, A., Pendry, B.A., Hughes, M.J., Webb, G.P. and Corcoran, O. 2008. Validation of a HPLC method for flavonoid biomarkers in skullcap (*Scutellaria*) and its use to illustrate wide variability in the quality of commercial tinctures. J. Pharm. Pharm. Sci. 11: 77–87.

Gavamukulya, Y., Abou-Elella, F., Wamunyokoli, F. and AEl-Shemy, H. 2014. Phytochemical screening, anti-oxidant activity and *in vitro* anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). Asian Pac. J. Trop. Med. 7: S355–S363.

Goldstein, D.A., Clark, J., Tu, Y., Zhang, J., Fang, F., Goldstein, R.M., Stemmer, S.M. and Rosenbaum, E. 2016. Global differences in cancer drug prices: a comparative analysis. J. Clin. Oncol. 34: LBA6500-LBA6500.

Gu, Z.M., Zhou, D., Lewis, N.J., Wu, J., Shi, G. and McLaughlin, J.L. 1997. Isolation of new bioactive annonaceous acetogenins from *Rollinia mucosa* guided by liquid chromatography/mass spectrometry. Bioorg. Med. Chem. 5: 1911–1916.

Guo, L., Chen, X., Hu, Y., Yu, Z., Wang, D. and Liu, J. 2013. Curcumin inhibits proliferation and induces apoptosis of human colorectal cancer cells by activating the mitochondria apoptotic pathway. Phytother. Res. 27: 422–430.

Gupta, M.P., Monge, A., Karikas, G.A., Lopez de Cerain, A., Solis, P.N., de Leon, E., Trujillo, M., Suarez, O., Wilson, F., Montenegro, G., Noriega, Y., Santana, A.I., Correa, M. and Sanchez, C. 1996. Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA intercalation. Int. J. Pharmacogn. 34: 19–27.

Han, S.-Y., Zhao, M.-B., Zhuang, G.-B. and Li, P.-P. 2012. *Marsdenia tenacissima* extract restored gefitinib sensitivity in resistant non-small cell lung cancer cells. Lung Cancer 75: 30–37.

Han, S.-Y., Ding, H.-R., Zhao, W., Teng, F. and Li, P.-P. 2014. Enhancement of gefitinib-induced growth inhibition by Marsdenia tenacissima extract in non-small cell lung cancer cells expressing wild or mutant EGFR. BMC Complement. Altern. Med. 14: 165.

Han, S.Y., Zhao, W., Sun, H., Zhou, N., Zhou, F., An, G. and Li, P.P. 2015. *Marsdenia tenacissima* extract enhances gefitinib efficacy in non-small cell lung cancer xenografts. Phytomedicine Int. J. Phytother. Phytopharm. 22: 560–567.

Harish Kumar, G., Chandra Mohan, K.V., Jagannadha Rao, A. and Nagini, S. 2009. Nimbolide a limonoid from *Azadirachta indica* inhibits proliferation and induces apoptosis of human choriocarcinoma (BeWo) cells. Invest. New Drugs 27: 246–252.

Hemamalini, K., Soujanya, G.L., Bhargav, A. and Vasireddy, U. 2012. *In-vivo* anticancer activity of *Tabebuia rosea* (Bertol) Dc. leaves on Dalton's ascetic lymphoma in mice. Int. J. Pharm. Sci. Res. 3: 4496–4502.

Hong, D.H., Bae Han, S., Lee, C., Hyung Park, S., Jin Jeon, Y., Kim, M.-J., Kwak, S.-S. and Kim, H. 1999. Cytotoxicity of urushiols isolated from sap of Korean lacquer tree (*Rhus vernicifera* Stokes). Arch. Pharm. Res. 22: 638–641.

Hu, Y.-J., Shen, X.-L., Lu, H.-L., Zhang, Y.-H., Huang, X.-A., Fu, L.-C. and Fong, W.-F. 2008. Tenacigenin B derivatives reverse P-glycoprotein-mediated multidrug resistance in HepG2/Dox cells. J. Nat. Prod. 71: 1049–1051.

Huang, S.-T., Wang, C.-Y., Yang, R.-C., Chu, C.-J., Wu, H.-T. and Pang, J.-H.S. 2010. Wogonin, an active compound in *Scutellaria baicalensis*, induces apoptosis and reduces telomerase activity in the HL-60 leukemia cells. Phytomedicine 17: 47–54.

Iwasa, K., Takahashi, T., Nishiyama, Y., Moriyasu, M., Sugiura, M., Takeuchi, A., Tode, C., Tokuda, H. and Takeda, K. 2008. Online structural elucidation of alkaloids and other constituents in crude extracts and cultured cells of *Nandina domestica* by combination of LC-MS/MS, LC-NMR, and LC-CD analyses. J. Nat. Prod. 71: 1376–1385.

Jagetia, G.C. and Baliga, M.S. 2016. Preclinical evaluation of the anticancer activity of hydroalcoholic stem bark extract of *Alstonia scholaris* in Ehrlich ascites carcinoma transplanted in the Swiss albino mice. J. Alt. Med. Res. 2: 115–126.

Jain, S.K. and Tarafder, C.R. 1970. Medicinal plant-lore of the santals (A revival of P. O. Bodding's work). Econ. Bot. 24: 241–278.

Jayaprakasam, B., Zhang, Y., Seeram, N.P. and Nair, M.G. 2003. Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. Life Sci. 74: 125–132.

Ji, S., Li, R., Wang, Q., Miao, W.-J., Li, Z.-W., Si, L., Qiao, X., Yu, S.-W., Zhou, D.-M. and Ye, M. 2015. Anti-H1N1 virus, cytotoxic and Nrf2 activation activities of chemical constituents from *Scutellaria baicalensis*. J. Ethnopharmacol. 176: 475–484.

Jovicevic, L., Troiani, M.P., Capezzone de Joannon, A., Saso, L., Mezzanti, G. and Rossi, V. 1993. *In vitro* antiproliferative activity of *Petiveria alliacea* L. on several tumor cell lines. Pharmacol. Res. 27: 105–106.

Jung, C.H., Kim, J.H., Hong, M.H., Seog, H.M., Oh, S.H., Lee, P.J., Kim, G.J., Kim, H.M., Um, J.Y. and Ko, S.-G. 2007. Phenolic-rich fraction from *Rhus verniciflua* Stokes (RVS) suppress inflammatory response via NF-κB and JNK pathway in lipopolysaccharide-induced RAW 264.7 macrophages. J. Ethnopharmacol. 110: 490–497.

Kim, H. and Song, M.-J. 2011. Analysis and recordings of orally transmitted knowledge about medicinal plants in the southern mountainous region of Korea. J. Ethnopharmacol. 134: 676–696.

Kim, K.H., Moon, E., Choi, S.U., Pang, C., Kim, S.Y. and Lee, K.R. 2015. Identification of cytotoxic and anti-inflammatory constituents from the bark of *Toxicodendron vernicifluum* (Stokes) F.A. Barkley. J. Ethnopharmacol. 162: 231–237.

Kim, M.-J., Choi, W.-C., Barshinikov, A.M. and Kobayashi, A. 2002. Anticancer and antioxidant activity of allergen-removed extract in *Rhus verniciflua* Stokes. Korean J. Med. Crop Sci. 10: 288–293.

Kumagai, T., Müller, C.I., Desmond, J.C., Imai, Y., Heber, D. and Koeffler, H.P. 2007. *Scutellaria baicalensis*, a herbal medicine: anti-proliferative and apoptotic activity against acute lymphocytic leukemia, lymphoma and myeloma cell lines. Leuk. Res. 31: 523–530.

Kuo, Y.H., Chen, C.H., Kuo, L.M.Y., King, M.L., Wu, T.S., Lu, S.T., Chen, I.S., McPhail, D.R., McPhail, A.T. and Lee, K.H. 1989. Structure and stereochemistry of emarginatine-A, a novel cytotoxic sesquiterpene pyridine alkaloid from *Maytenus emarginata*: X-ray crystal structure of emarginatine-A monohydrate. Heterocycles 29: 1465–1468.

Kuo, Y.-H., Chen, C.-H., Kuo, L.-M.Y., King, M.-L., Wu, T.-S., Haruna, M. and Lee, K.-H. 1990. Antitumor agents, 112. Emarginatine B, a novel potent cytotoxic sesquiterpene pyridine alkaloid from *Maytenus emarginata*. J. Nat. Prod. 53: 422–428.

Kuo, Y.-H., Chen, C.-H., King, M.-L., Wu, T.-S. and Lee, K.-H. 1994a. Sesquiterpene pyridine alkaloids from *Maytenus emarginata*: emarginatine-C and -D and cytotoxic emarginatine-E and emarginatinine. Phytochemistry 35: 803–807.

Kuo, Y.-H., King, M.-L., Chen, C.-F., Chen, H.-Y., Chen, C.-H., Chen, K. and Lee, K.-H. 1994b. Two new macrolide sesquiterpene pyridine alkaloids from *Maytenus emarginata*: emarginatine G and the cytotoxic emarginatine F. J. Nat. Prod. 57: 263–269.

Kuttan, R., Bhanumathy, P., Nirmala, K. and George, M.C. 1985. Potential anticancer activity of turmeric (*Curcuma longa*). Cancer Lett. 29: 197–202.

Lau, S.T., Lin, Z.-X., Zhao, M. and Leung, P.S. 2008. *Brucea javanica* fruit induces cytotoxicity and apoptosis in pancreatic adenocarcinoma cell lines. Phytother. Res. 22: 477–486.

Le, P.M., Srivastava, V., Nguyen, T.T., Pradines, B., Madamet, M., Mosnier, J., Trinh, T.T. and Lee, H. 2017. Stephanine from *Stephania venosa* (Blume) Spreng showed effective antiplasmodial and anticancer activities, the latter by inducing apoptosis through the reverse of mitotic exit. Phytother. Res. 31: 1357–1368.

Lee, J.-W., Kim, K.-S., An, H.-K., Kim, C.-H., Moon, H.-I. and Lee, Y.-C. 2013. Dendropanoxide induces autophagy through ERK1/2 activation in MG-63 human osteosarcoma cells and autophagy inhibition enhances dendropanoxide-induced apoptosis. PLoS ONE 8: e83611.

Leewanich, P., Worachartcheewan, A., Prachayasittikul, S. and Prachayasittikul, V. 2011. Anticancer and antioxidative activities of *Stephania venosa*. Eur. J. Sci. Res. 51: 150–156.

Levine, A.J., Reich, N. and Thomas, R. 1983. The regulation of a cellular protein, p53, in normal and transformed cells. Prog. Clin. Biol. Res. 119: 159–169.

Li, J., Jadhav, A.N., Rumalla, C.S. and Khan, I.A. 2009. New terpenoids from *Pfaffia paniculata* Kuntze. Planta Med. 75: 37.
Li, J., Lei, X. and Chen, K. 2014a. Comparison of cytotoxic activities of extracts from *Selaginella* species. Pharmacogn. Mag. 10: 529–535.
Li, J., Zhang, L., Huang, C., Guo, F. and Li, Y. 2014b. Five new cyotoxic steroidal glycosides from the fruits of *Solanum torvum*. Fitoterapia 93: 209–215.
Li, X.-H., Hui, Y.-H., Rupprecht, J.K., Liu, Y.-M., Wood, K.V., Smith, D.L., Chang, C.-J. and McLaughlin, J.L. 1990. Bullatacin, bullatacinone, and squamone, a new bioactive acetogenin, from the bark of *Annona squamosa*. J. Nat. Prod. 53: 81–86.
Lin, C.-C. and Lin, C.-H. 1993. Pharmacological and pathological studies on Taiwan folk medicine (IX): the hepatoprotective effect of the methanolic extract from *Echinops grijisii*. Am. J. Chin. Med. 21: 33–44.
Lin, Y.-L., Huang, R.-L., Chang, C.-M. and Kuo, Y.-H. 1997. Two new puriniums and three new pyrimidines from *Heterostemma brownii*. J. Nat. Prod. 60: 982–985.
Ling, H.-C., King, M.-L., Su, M.-H., Chen, G.-L. and Wang, C.-T. 1981. Study on antitumor plant *Gymnosporia trilocularis*. J. Chin. Chem. Soc. 28: 95–101.
Luo, S.-Q., Lin, L.-Z., Cordell, G.A., Xue, L. and Johnson, M.E. 1993. Polyoxypregnanes from *Marsdenia tenacissima*. Phytochemistry 34: 1615–1620.
Malek, S.N.A., Lee, G.S., Hong, S.L., Yaacob, H., Wahab, N.A., Faizal Weber, J.-F. and Shah, S.A.A. 2011. Phytochemical and cytotoxic investigations of *Curcuma mangga* rhizomes. Molecules 16: 4539–4548.
Mata, R., Rivero-Cruz, J.F. and Chávez, D. 2001. Bioactive secondary metabolites from selected Mexican medicinal plants: recent progress. In: Bioactive compounds from natural sources: isolation, characterization and biological properties. Taylor and Francis Group, New York.
MEDS - Medical Expenditure Panel Survey from AHRQ - Agency for Healthcare Research and Quality. Summary Data Tables Search Results. 2014. https://meps.ahrq.gov/data_stats/quick_tables_results.jsp?component=1&subcomponent=0&tableSeries=2&year=-1&SearchMethod=1&Action=Search (accessed 8.11.17).
Mena-Rejón, G., Caamal-Fuentes, E., Cantillo-Ciau, Z., Cedillo-Rivera, R., Flores-Guido, J. and Moo-Puc, R. 2009. *In vitro* cytotoxic activity of nine plants used in Mayan traditional medicine. J. Ethnopharmacol. 121: 462–465.
Momekova, D., Momekov, G., Pencheva, I. and Konstantinov, S. 2012. Antineoplastic activity of a methanolic extract from *Kigelia pinnata* DC stem bark. J. Cancer Ther. Res. 1: 17 (8 p.).
Monteiro, L.S., Bastos, K.X., Barbosa-Filho, J.M., de Athayde-Filho, P.F., Diniz, M.F.F.M. and Sobral, M.V. 2014. Medicinal plants and other living organisms with antitumor potential against lung cancer. Evid. Based Complement. Alternat. Med. 2014: 604152 (15 p.).
Naama, J.H., Al-Temimi, A.A. and Hussain Al-Amiery, A.A. 2010. Study the anticancer activities of ethanolic curcumin extract. Afr. J. Pure Appl. Chem. 4: 68–73.
Nantapap, S., Loetchutinat, C., Meepowpan, P., Nuntasaen, N. and Pompimon, W. 2010. Antiproliferative effects of alkaloids isolated from the tuber of *Stephania venosa* via the induction of cell cycle arrest in mammalian cancer cell lines. Am. J. Appl. Sci. 7: 1057–1065.
NCI - National Cancer Institute. 2017. Cancer statistics. https://www.cancer.gov/about-cancer/understanding/statistics (accessed 8.11.17).
Nema, R., Khare, S., Jain, P. and Pradhan, A. 2013. Anticancer activity of *Withania somnifera* (leaves) flavonoids compound. Int. J. Pharm. Sci. Rev. Res. 19: 103–106.
Neves, M.P., Cidade, H., Pinto, M., Silva, A.M., Gales, L., Damas, A.M., Lima, R.T., Vasconcelos, M.H. and de São José Nascimento, M. 2011. Prenylated derivatives of baicalein and 3,7-dihydroxyflavone: synthesis and study of their effects on tumor cell lines growth, cell cycle and apoptosis. Eur. J. Med. Chem. 46: 2562–2574.
Ni, G., Zhang, H., Liu, H.-C., Yang, S.-P., Geng, M.-Y. and Yue, J.-M. 2013. Cytotoxic sesquiterpenoids from *Sarcandra glabra*. Tetrahedron 69: 564–569.
Niwat, K., Itharat, A., Aranya, T., Suttipong, R. and Preecha, I. 2001. Cytotoxic alkaloids from the tuber of *Stephania venosa*. J. Sci. Technol. 23: 225–234.
Nopsiri, W., Chansakaow, S., Putiyanan, S., Natakankitkul, S. and Santiarworn, D. 2014. Antioxidant and anticancer activities from leaf extracts of four *Combretum* species from Northern Thailand. CMU J. Nat. Sci. 13: 195–205.
Oliveira, V.B., Freitas, M.S.M., Mathias, L., Braz-Filho, R. and Vieira, I.J.C. 2009. Biological activity and indole alkaloids of the genus *Aspidosperma* (Apocynaceae): a review. Rev. Bras. Plantas Med. 11: 92–99.
Pan, X., Matsumoto, M., Nakamura, Y., Kikuchi, T., Zhang, J., Ukiya, M., Suzuki, T., Koike, K., Akihisa, R. and Akihisa, T. 2014. Three new and other limonoids from the hexane extract of *Melia azedarach* fruits and their cytotoxic activities. Chem. Biodivers 11: 987–1000.
Park, Y., Han, J., Lee, T. and Kim, D. 2005. Growth inhibitory and antioxidative effects of crude methanolic extract from *Euonymus alatus* (Thunb.) Sieb on SKBR3 human breast cancer cell line. J. Orient. Obstet. Gynecol. 18: 45–54.
Paul, R., Prasad, M. and Sah, N.K. 2011. Anticancer biology of *Azadirachta indica* L. (neem): a mini review. Cancer Biol. Ther. 12: 467–476.
Pecere, T., Gazzola, M.V., Mucignat, C., Parolin, C., Vecchia, F.D., Cavaggioni, A., Basso, G., Diaspro, A., Salvato, B., Carli, M. and Palù, G. 2000. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. Cancer Res. 60: 2800–2804.
Peng, C.-Y., Liu, J.-Q., Zhang, R. and Shu, J.-C. 2014. A new alkaloid from the fruit of *Nandina domestica* Thunb. Nat. Prod. Res. 28: 1159–1164.

Perdue, G.P. and Blomster, R.N. 1978. South American plants III: Isolation of fulvoplumierin from *Himatanthus sucuuba* (M. Arg.) Woodson (Apocynaceae). J. Pharm. Sci. 67: 1322–1323.

Priyadarsini, R.V., Murugan, R.S., Sripriya, P., Karunagaran, D. and Nagini, S. 2010. The neem limonoids azadirachtin and nimbolide induce cell cycle arrest and mitochondria-mediated apoptosis in human cervical cancer (HeLa) cells. Free Radic. Res. 44: 624–634.

Rai, M., Jogee, P.S., Agarkar, G. and dos Santos, C.A. 2016. Anticancer activities of *Withania somnifera*: current research, formulations, and future perspectives. Pharm. Biol. 54: 189–197.

Ravipati, A.S., Zhang, L., Koyyalamudi, S.R., Jeong, S.-C., Reddy, N., Bartlett, J.R., Smith, P.T., de Pedro, N., Melguizo, A., Cantizani, J., Asensio, F. and Vicente, F. 2013. Anti-proliferative activities of selected Chinese medicinal herbs against human cancer cell lines. Phytopharmacology 4: 206–219.

Réthy, B., Csupor-Löffler, B., Zupkó, I., Hajdú, Z., Máthé, I., Hohmann, J., Rédei, T. and Falkay, G. 2007. Antiproliferative activity of Hungarian Asteraceae species against human cancer cell lines. Part I. Phytother. Res. 21: 1200–1208.

Rodríguez, F.M., López, M.R., Jiménez, I.A., Moujir, L., Ravelo, A.G. and Bazzocchi, I.L. 2005. New phenolic triterpenes from *Maytenus blepharodes*. Semisynthesis of 6-deoxoblepharodol from pristimerin. Tetrahedron 61: 2513–2519.

Rossi, V., Jovicevic, L., Troiani, M.P., Bonanomi, M. and Mazzanti, G. 1990. Antiproliferative effects of *Petiveria alliacea* on several tumor cell lines. Pharmacol. Res. 22(Suppl. 2): 434.

Rossi, V., Marini, S., Jovicevic, L., D'Atri, S., Turri, M. and Giardina, B. 1993. Effects of *Petiveria alliacea* L. on cell immunity. Pharmacol. Res. 27(Suppl. 1): 111–112.

Roy, M.K., Kobori, M., Takenaka, M., Nakahara, K., Shinmoto, H., Isobe, S. and Tsushida, T. 2007. Antiproliferative effect on human cancer cell lines after treatment with nimbolide extracted from an edible part of the neem tree (*Azadirachta indica*). Phytother. Res. 21: 245–250.

Sagwan, S., Rao, D.V. and Sharma, R.A. 2011. *In vitro* antioxidant activity of *Maytenus emarginata* (Willd.) using DPPH assay. J. Pharm. Res. 4: 2276–2278.

Scheck, A.C., Perry, K., Hank, N.C. and Clark, W.D. 2006. Anticancer activity of extracts derived from the mature roots of *Scutellaria baicalensis* on human malignant brain tumor cells. BMC Complement. Altern. Med. 6: 27 (9 p.).

Schmidt, B.M., and Cheng, D.M.K. 2017. Ethnobotany: A phytochemical Perspective. John Wiley & Sons, Hoboken.

Shao, Z.-M., Shen, Z.-Z., Liu, C.-H., Sartippour, M.R., Go, V.L., Heber, D. and Nguyen, M. 2002. Curcumin exerts multiple suppressive effects on human breast carcinoma cells. Int. J. Cancer 98: 234–240.

Sharma, J.V., Pitchaiah, G., Satyavati, D., Rao, J., Kumar, H. and Sanjay, H. 2011. *In vitro* anticancer of methanolic extract of roots of *Glochidion zeylanicum* (Gaertn). Int. J. Pharma. Bio. Sci. 2: 760–764.

Shi, G., MacDougal, J.M. and McLaughlin, J.L. 1997. Bioactive annonaceous acetogenins from *Rollinia mucosa*. Phytochemistry 45: 719–723.

Shi, G., Ye, Q., He, K., McLaughlin, J.L. and MacDougal, J.M. 1996. Rollinecins A and B: two new bioactive annonaceous acetogenins from *Rollinia mucosa*. J. Nat. Prod. 59: 548–551.

Shi, M., Cai, Q., Yao, L., Mao, Y., Ming, Y. and Ouyang, G. 2006. Antiproliferation and apoptosis induced by curcumin in human ovarian cancer cells. Cell Biol. Int. 30: 221–226.

Sichaem, J., Kaennakam, S., Siripong, P. and Tip-pyang, S. 2012. Tabebuialdehydes A-C, cyclopentene dialdehyde derivatives from the roots of *Tabebuia rosea*. Fitoterapia 83: 1456–1459.

Singleterry, J. 2017. The costs of cancer. Available on-line https://www.acscan.org/policy-resources/costs-cancer.

Su, S., Wang, T., Chen, T., Duan, J., Yu, L. and Tang, Y. 2011. Cytotoxicity activity of extracts and compounds from *Commiphora myrrha* resin against human gynecologic cancer cells. J. Med. Plants Res. 5: 1382–1389.

Sultana, S., Asif, H.M., Nazar, H.M., Akhtar, N., Rehman, J.U. and Rehman, R.U. 2014. Medicinal plants combating against cancer—a green anticancer approach. Asian Pac. J. Cancer Prev. 15: 4385–4394.

Takagi, M., Tachi, Y., Zhang, J., Shinozaki, T., Ishii, K., Kikuchi, T., Ukiya, M., Banno, N., Tokuda, H. and Akihisa, T. 2014. Cytotoxic and melanogenesis-inhibitory activities of limonoids from the leaves of *Azadirachta indica* (Neem). Chem. Biodivers 11: 451–468.

Takemoto, T., Nishimoto, N., Nakai, S., Takagi, N., Hayashi, S., Odashima, S. and Wada, Y. 1983. Pfaffic acid, a novel nortriterpene from *Pfaffia paniculata* Kuntze. Tetrahedron Lett. 24: 1057–1060.

Tan, M., Sulaiman, S. and Najimudin, N. 2007. Anticancer medicinal plant, *Epipremnum pinnatum* (L.) Engl. chloroform extracts elicited both apoptotic and non-apoptotic cell deaths in T-47D mammary carcinoma cells. KMITL Sci. Tech. J. 7: 24–43.

Tan, M.L., Muhammad, T.S.T., Najimudin, N. and Sulaiman, S.F. 2005. Growth arrest and non-apoptotic programmed cell death associated with the up-regulation of c-myc mRNA expression in T-47D breast tumor cells following exposure to *Epipremnum pinnatum* (L.). Engl. hexane extract. J. Ethnopharmacol. 96: 375–383.

Tsui, W.-Y. and Brown, G.D. 1996. Cycloeudesmanolides from *Sarcandra glabra*. Phytochemistry 43: 819–821.

Tzeng, C.C., Wu, Y.C., Su, T.L., Watanabe, K.A., Lu, S.T. and Chou, T.C. 1990. Inhibitory effects of isoquinoline-type alkaloids on leukemic cell growth and macromolecule biosynthesis. Kaohsiung J. Med. Sci. 6: 58–65.

Ukiya, M., Akihisa, T., Yasukawa, K., Tokuda, H., Suzuki, T. and Kimura, Y. 2006. Anti-inflammatory, anti-tumor-promoting, and cytotoxic activities of constituents of marigold (*Calendula officinalis*) flowers. J. Nat. Prod. 69: 1692–1696.

Vega-Ávila, E., Tapia-Aguilar, R., Jiménez-Estrada, M., Villareal, M.L. and Ramos, R.R. 2004. Cytotoxic activity of *Cuphea aequipetala*. Proc. West. Pharmacol. Soc. 47: 129–133.

Wang, W., Zhao, Y., Rayburn, E.R., Hill, D.L., Wang, H. and Zhang, R. 2007. *In vitro* anti-cancer activity and structure-activity relationships of natural products isolated from fruits of *Panax ginseng*. Cancer Chemother. Pharmacol. 59: 589–601.

Weissenstein, U., Kunz, M., Urech, K., Regueiro, U. and Baumgartner, S. 2016. Interaction of a standardized mistletoe (*Viscum album*) preparation with antitumor effects of Trastuzumab *in vitro*. BMC Complement. Altern. Med. 16: 271 (10 p.).

Widowati, W., Mozef, T., Risdian, C. and Yellianty, Y. 2013. Anticancer and free radical scavenging potency of *Catharanthus roseus*, *Dendrophthoe petandr*a, *Piper betle* and *Curcuma mangga* extracts in breast cancer cell lines. Oxid. Antioxid. Med. Sci. 2: 137–142.

Wong, K.T. and Tan, B.K.H. 1996a. *In vitro* cytotoxicity and immunomodulating property of *Rhaphidophora korthalsii*. J. Ethnopharmacol. 52: 53–57.

Wong, K.T., Tan, B.K.H., Sim, K.Y. and Goh, S.H. 1996b. A cytotoxic melanin precursor, 5,6-dihydroxyindole, from the folkloric anti-cancer plant *Rhaphidophora korthalsii*. Nat. Prod. Lett. 9: 137–140.

WHO. 2015. Globocan 2012: Estimated Incidence, Mortality and Prevalence Worldwide in 2012.

Wu, F.E., Gu, Z.M., Zeng, L., Zhao, G.X., Zhang, Y., McLaughlin, J.L. and Sastrodihardjo, S. 1995. Two new cytotoxic monotetrahydrofuran Annonaceous acetogenins, annomuricins A and B, from the leaves of *Annona muricata*. J. Nat. Prod. 58: 830–836.

Wu, J.-B., Cheng, Y.-D., Chiu, N.-Y., Huang, S.-C. and Kuo, S.-C. 1993. A novel morphinandienone alkaloid from *Fissistigma oldhamii*. Planta Med. 59: 179–180.

Wu, J.-T., Lu, S.M., Lu, C.-H., Gong, J. and An, J.-B. 2015. Effect of 3,3'-biisofraxidin on apoptosis of human gastric cancer BGC-823 cells. Trop. J. Pharm. Res. 14: 1803–1811.

Wu, Q., Kohli, M., Bergen, H.R., Cheville, J.C., Karnes, R.J., Cao, H., Young, C.Y., Tindall, D.J., McNiven, M.A. and Donkena, K.V. 2014. Preclinical evaluation of the supercritical extract of *Azadirachta indica* (neem) leaves *in vitro* and *in vivo* on inhibition of prostate cancer tumor growth. Mol. Cancer Ther. 13: 1067–1077.

Yamashita, M., Kaneko, M., Iida, A., Tokuda, H. and Nishimura, K. 2007. Stereoselective synthesis and cytotoxicity of a cancer chemopreventive naphthoquinone from *Tabebuia avellanedae*. Bioorg. Med. Chem. Lett. 17: 6417–6420.

Yamashita, M., Kaneko, M., Tokuda, H., Nishimura, K., Kumeda, Y. and Iida, A. 2009. Synthesis and evaluation of bioactive naphthoquinones from the Brazilian medicinal plant, *Tabebuia avellanedae*. Bioorg. Med. Chem. 17: 6286–6291.

Yang, H.-S., Kim, J.-Y., Lee, J.-H., Lee, B.-W., Park, K.-H., Shim, K.-H., Lee, M.-K. and Seo, K.-I. 2011. Celastrol isolated from *Tripterygium regelii* induces apoptosis through both caspase-dependent and -independent pathways in human breast cancer cells. Food Chem. Toxicol. 49: 527–532.

Yen, C.-Y., Chiu, C.-C., Chang, F.-R., Chen, J.Y.-F., Hwang, C.-C., Hseu, Y.-C., Yang, H.-L., Lee, A.Y.-L., Tsai, M.-T., Guo, Z.-L., Cheng, Y.-S., Liu, Y.-C., Lan, Y.-H., Chang, Y.-C., Ko, Y.-C., Chang, H.-W. and Wu, Y.-C. 2010. 4β-Hydroxywithanolide E from *Physalis peruviana* (golden berry) inhibits growth of human lung cancer cells through DNA damage, apoptosis and G2/M arrest. BMC Cancer 10: 46 (8 p.).

Zeng, L., Wu, F.-E., Oberlies, N.H., McLaughlin, J.L. and Sastrodihadjo, S. 1996. Five new monotetrahydrofuran ring acetogenins from the leaves of *Annona muricata*. J. Nat. Prod. 59: 1035–1042.

Zhang, L., Tatsuno, T., Hasegawa, I., Tadano, T. and Ohta, T. 2015. Furanonaphthoquinones from *Tabebuia avellanedae* induce cell cycle arrest and apoptosis in the human non-small cell lung cancer cell line A549. Phytochem. Lett. 11: 9–17.

Zhang, P., Liang, D., Jin, W., Qu, H., Cheng, Y., Li, X. and Ma, Z. 2009. Cytotoxic thiophenes from the root of *Echinops grijisii* Hance. Z. Naturforsch. C 64: 193–196.

Zhao, W., Entschladen, F., Liu, H., Niggemann, B., Fang, Q., Zaenker, K.S. and Han, R. 2003. Boswellic acid acetate induces differentiation and apoptosis in highly metastatic melanoma and fibrosarcoma cells. Cancer Detect. Prev. 27: 67–75.

Zhao, W., Wang, Y., Wang, Y., Gao, N., Han, Z. and Yu, H. 2015. Potential anti-cancer effect of curcumin in human lung squamous cell carcinoma. Thorac. Cancer 6: 508–516.

Zhou, S., Yao, D., Guo, L. and Teng, L. 2017. Curcumin suppresses gastric cancer by inhibiting gastrin-mediated acid secretion. FEBS Open Bio. 7: 1078–1084.

2

Use of Ethnomedicinal Herbs to Treat and Manage Schistosomiasis in Zimbabwe: Past Trends and Future Directions

Alfred Maroyi

Introduction

Schistosomiasis also known as bilharzia is a freshwater snail transmitted intravascular debilitating disease resulting from infection by the parasitic dimorphic *Schistosoma* trematode worms which live in the bloodstream of humans (Steinmann et al. 2006, Inobaya et al. 2014, Adenowo et al. 2015, WHO 2017a). Schistosomiasis is a neglected tropical disease that ranks second only to malaria in terms of human suffering in the tropics and subtropics (Inobaya et al. 2014). Reports by the World Health Organization (WHO 2017a) showed that at least 218 million people required preventive treatment for schistosomiasis and more than 66.5 million people were reported to have been treated for the disease in 2015. Schistosomiasis transmission has been reported from 78 countries while preventive chemotherapy for schistosomiasis is required in 52 countries where the disease is endemic with moderate-to-high transmission (WHO 2017a). According to WHO (2017b), schistosomiasis causes more than 200,000 deaths per year in sub-Saharan Africa. Previous reports by Adenowo et al. (2015) revealed that sub-Saharan Africa accounted for 93% (192 million) of the world estimated 207 million cases of schistosomiasis in 2014 with the highest prevalence of this infection recorded in Nigeria (29 million), followed by Tanzania (19 million), Ghana and Democratic Republic of Congo with 15 million each and Mozambique with 13 million. Schistosomiasis causes great health, social and financial burden on economies of households and governments in sub-Saharan Africa with profound negative effects on child development, outcome of pregnancy, and agricultural productivity (Adenowo et al. 2015). Schistosomiasis is more rampant in poor and marginalized rural communities where fishing and agricultural activities are dominant (Adenowo et al. 2015, WHO 2017a). Women and children

Medicinal Plants and Economic Development (MPED) Research Center, Department of Botany, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa.
E-mail: amaroyi@ufh.ac.za

doing domestic chores such as washing clothes and fetching water in infected water sources are at risk as inadequate hygiene and contact with infected water expose such people to schistosomiasis infection.

There are two major forms of schistosomiasis, namely intestinal and urogenital caused by five digenean blood flukes of the genus *Schistosoma* (WHO 2017a). *Schistosoma haematobium* causes urogenital schistosomiasis in Africa, the Middle East and Corsica in France (WHO 2017a) affecting about 54 countries in total (Ojewole 2004). The other four *Schistosoma* species are responsible for intestinal schistosomiasis with *Schistosoma mansoni* distributed in Africa, Brazil, the Caribbean, the Middle East, Suriname and Venezuela, *Schistosoma japonicum* (China, Indonesia and the Philippines), *Schistosoma mekongi* (several districts in Cambodia and Lao People's Democratic Republic), *Schistosoma guineensis* and closely related *Schistosoma intercalatum* (confined to the rain forest areas of central Africa) (WHO 2017a). Schistosomiasis is spread when the larvae of *Schistosoma* species are liberated by the infected snail, intermediary host, get in contact with the human host and subsequently penetrate the skin. Therefore, in humans, schistosomiasis is spread through skin contact with fresh water containing infectious larvae of *Schistosoma* species. *Biomphalaria* snails are responsible for the transmission of *Schistosoma mansoni*, *Bulinus* snails transmit *Schistosoma haematobium* while *Schistosoma japonicum* is spread by the freshwater snail *Oncomelania* (Adenowo et al. 2015). Once inside the human body, the pathogens differentiate into schistosomules, which migrate via the bloodstream to the liver and develop into male and female mature forms (Ndjonka et al. 2013). After mating, the worms migrate again and relocate at the mesenteric intestinal veins or the venous plexus of the urinary system. The females release eggs, which are able to pass epithel of the blood vessels and reach the intestinal lumen, the bladder or urethra lumen in order to be expelled by faeces or urine. Some of these eggs also remain in these tissues and the damage of blood vessels, together with immune reactions against the retained eggs are responsible for the clinical forms of schistosomiasis (Ndjonka et al. 2013). According to WHO (2017a), intestinal schistosomiasis can result in abdominal pain, diarrhoea, and blood in the stool. Liver enlargement is common in advanced cases, and is frequently associated with an accumulation of fluid in the peritoneal cavity and hypertension of the abdominal blood vessels. The classic sign of urogenital schistosomiasis is haematuria (blood in urine), fibrosis of the bladder and ureter, and kidney damage are sometimes diagnosed in advanced cases of urogenital schistosomiasis (WHO 2017a). Bladder cancer is another possible complication in the later stages of urogenital schistosomiasis. In women, urogenital schistosomiasis may present with genital lesions, vaginal bleeding, pain during sexual intercourse and nodules in the vulva. In men, urogenital schistosomiasis can induce pathology of the seminal vesicles, prostate and other organs. This disease may also have other long-term irreversible consequences such as infertility in both men and women (WHO 2017a). The economic and health effects of schistosomiasis are considerable and the disease disables more than it kills. In children, schistosomiasis can cause anaemia, stunting and a reduced ability to learn, although the effects are usually reversible with treatment and chronic schistosomiasis may affect people's ability to work and in some cases can result in death (WHO 2017a).

Schistosomiasis in Zimbabwe

Before the advent of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS), schistosomiasis was ranked second after malaria in terms of public health importance in Zimbabwe (Chimbari 2012). Schistosomiasis has for several years been among the top 10 causes of hospital admissions in Zimbabwe, an indication of its public health importance (Chimbari 2012). Both urinary and intestinal schistosomiasis are endemic in Zimbabwe, caused by *Schistosoma haematobium* and *Schistosoma mansoni* respectively. The disease is widespread throughout the country in both rural and urban areas with *Schistosoma haematobium* more widespread than *Schistosoma mansoni* at prevalences of 18.0% and 7.2% respectively (Midzi et al. 2014). In Zimbabwe, *Schistosoma haematobium* and *Schistosoma mansoni* are transmitted by the intermediate snail hosts *Bulinus globosus* and *Biomphalaria pfeifferi* respectively (Pedersen et al. 2017) with both snail species common in most bodies of fresh water in the country. Schistosomiasis control in Zimbabwe included control of intermediate host snails *Biomphalaria pfeifferi* and *Bulinus globosus* and treatment based approach (Chimbari 2012), see Table 2.1.

Table 2.1 Schistosomiasis control in Zimbabwe.

Control model	Relevant notes
Two plant-based molluscides (*Phytolacca dodecandra* L'Hér. and *Jatropha curcas* L.) have been studied with a view to use them in preference to the WHO recommended molluscicide, niclosamide	*Phytolacca dodecandra* has been studied in sufficient detail to justify its application while *Jatropha curcas* studies were only done in the laboratory (Chimbari 2012). The potency of water extracts of *Jatropha curcas* is much lower (75 ppm) compared to that of *Phytolacca dodecandra* (10 ppm) implying that larger quantities of the former would be required to sustain snail control activities. This control model has been characterized by low level community participation, poor leadership, low economic value of the plant, inaccessible fields, and lack of tangible benefits (Chimbari 2012). Other plant species that have been used for schistosomiasis control in Zimbabwe with known molluscicidal properties include *Combretum imberbe* Wawra, *Ricinus communis* L., *Trichilia emetica* Vahl, *Vernonia amygdalina* Delile and *Ximenia caffra* Sond (Ojewole 2004).
Ducks were introduced as a strategy to control intermediate host snails for schistosomiasis	According to Chimbari (2012) ducks made significant impact in reducing snail numbers in ponds but the costs associated with transportation of the ducks and looking after them to avoid poaching were high. Furthermore, the breeding and maintenance costs of the ducks were high as they were exotic species (Chimbari 2012).
Fish (*Sargochromis codringtonii*) was introduced as a strategy to control intermediate host snails for schistosomiasis	Comprehensive studies showed that pulmonates and not necessarily intermediate host snails were preferred by the fish (*Sargochromis codringronii*) and that vegetation provided refugia for snails against the predator fish (Chimbari 2012). *Sargochromis codringronii* was often attacked by a fish herbivore (*Tilapia rendalli*), and *Sargochromis codringronii* could only acclimatize to small ponds (100 m X 100 m X 1–1.5 m depth) in some regions of the country (Chimbari 2012).
Competitor snail (*Bulinus tropicus*) was introduced as a strategy to control intermediate host snails for schistosomiasis	Laboratory studies showed significant reduction in reproductivity of *Bulinus globosus* in the presence of the competitor snail (*Bulinus tropicus*) and evidence of *Bulinus tropicus* preying on *Bulinus globosus* eggs, but further enclosure studies did not show any significant effect of *Bulinus tropicus* on *Bulinus globosus* population density suggesting the competition between the two snail species was not an important control strategy of schistosomiasis in Zimbabwe (Chimbari 2012).

Use of praziquantel

Praziquantel (PZQ) is the drug of choice for the treatment of schistosomiasis in Zimbabwe. According to Magnussen (2003), a single dose of 40 mg/kg has been widely accepted as the standard dosage resulting in cure rates of 60 to 95%. Research done by Doenhoff et al. (2008) and Aragon et al. (2009) revealed that PZQ is not 100% curative in killing adult worms, cannot kill migrating schistosomulae or the early stages of the disease and does not prevent re-infection. Until recently, children aged five years and below were excluded from schistosome treatment using PZQ, creating a health inequity in affected populations (Mutapi et al. 2011). Research by Mutapi et al. (2011) revealed that PZQ treatment is as safe and efficacious in children aged one to five years as it is in older children in whom PZQ is the drug of choice for control of schistosome infections. In affected populations, children carry the heaviest burden of schistosome infection (Gryseels and de Vlas 1996, Midzi et al. 2008, Mutapi et al. 2011) with urogenital schistosomiasis causing haematuria, dysurea, nutritional deficiencies, anaemia, growth retardation, decreased physical performance and impaired memory and cognition in young children (Mutapi et al. 2011). Control of schistosome infections is through treatment of infected people with a single dose of the anthelmintic drug PZQ which is safe, cheap (costing less than US$0.50/dose) and can reverse schistosome-related morbidity particularly in the early stages of disease progression (King 2006, Mutapi et al. 2011). In South Africa, only Bayer's Biltricide® (PZQ) is available for the treatment of schistosomiasis at a cost of US$4.49 per tablet making mass treatment programmes unaffordable and almost impossible to run in the country (Magaisa et al. 2015). Research by Magaisa et al. (2015) revealed that PZQ is not fully stocked in local clinics in South Africa as the drug is considered to be too expensive. In 2010, the WHO updated their recommendations for the treatment of schistosomiasis in children aged five years and below, allowing regular pre-school based deworming using PZQ, aimed at reducing morbidity and promoting child health (Mutapi 2015).

Previous research by Midzi et al. (2008) and Mutapi et al. (2011) showed that PZQ usage in Zimbabwe is efficacious, with schistosome cure and egg reduction rates typically greater than 90%.

According to Ross et al. (2017), the PZQ strategy of eliminating schistosomiasis is failing and will not lead to disease elimination as only 5% of the reservoir human population is actually receiving intermittent chemotherapy. This strategy is failing because the drugs are not getting to the people who need them the most, the current global coverage is 20%, the drug compliance rate is less than 50% and the drug efficacy is approximately 50% (Ross et al. 2017). Adverse effects associated with PZQ usage including fatigue, urticaria, gastrointestinal and abdominal pains, nausea, vomiting, headache and dizziness (Mutapi 2015). Researchers such as Neves et al. (2015) are concerned about reliance on a single PZQ drug for treating and managing schistosome infections which are known to be affecting 249 million people. Praziquantel is now facing the threat of drug resistance as revealed by both laboratory and field trials (Fallon and Doenhoff 1994, Ismail et al. 1994, Fallon et al. 1995, Ismail et al. 1996, Melman et al. 2009, Couto et al. 2011). These reports of PZQ resistance indicates the need for new effective compounds to treat and manage schistosome infections, and globally, there is renewed interest in natural products as a starting point for drug discovery and development for schistosome infections (Ndjonka et al. 2013, Neves et al. 2015).

Potential of herbal medicines in treating and managing schistosomiasis in Zimbabwe

The only control method of schistosomiasis in Zimbabwe that has been successful is the use of PZQ. Other strategies meant to reduce schistosome infection include educating the public about sanitation, use of clean water and avoiding infected water bodies. The interest in medicinal plants used as herbal medicines for shistosomiasis or bilharzia (Gelfand et al. 1985, Hedberg and Staugard 1989, Ndamba et al. 1994, Hutchings et al. 1996, Mavi 1996, Clark et al. 1997, Sparg et al. 2000, Mølgaard et al. 2001, Maroyi 2011, Aremu et al. 2012) and PZQ developing drug resistances (Fallon and Doenhoff 1994, Ismail et al. 1994, Fallon et al. 1995, Ismail et al. 1996, Melman et al. 2009, Couto et al. 2011), and the limited access of poor communities and those in marginalized areas to PZQ have stimulated renewed interest in the current use and future potential of plant products in treating schistosomiasis, both as part of traditional health care practices and in developing new conventional medicines. In this study, 35 plant species belonging to 16 families and 32 genera are known to be widely used in the treatment and management of schistosomiasis in Zimbabwe (Table 2.2). Plant species recorded in Table 2.2 have been cited by at least two independent researchers as herbal medicines for schistosomiasis or cited by at least one researcher but are also known to have anthelmintic bioactivity based on *in vitro* or *in vivo* studies. The majority of these plant species (65.7%) are from four families, Fabaceae with 13 species, followed by Anacardiaceae with four species, and Asteraceae and Combretaceae families with three species each. The rest of the plant families are represented by a single species each, and the most common genera are *Lannea*, *Terminalia* and *Vernonia* with two species each (Table 2.2).

Shrubs (42.8%) and trees (40.0%) appear to be the primary sources of herbal medicines used for treating and managing schistosomiasis in Zimbabwe (Fig. 2.1A). The roots are the most frequently used plant parts (91.4%), followed by the bark (22.9%) and leaves (14.3%) (Fig. 2.1B). All plant remedies are usually utilized in the form of extracts and taken orally (Table 2.2). Monotherapy preparations made from a single plant species are the most dominant (74.3%) form of herbal preparations (Table 2.2). Apart from roots of *Elephantorrhiza goetzei* (Harms) Harms which are mixed with those of *Piliostigma thonningii* (Schumach.) Milne-Redh. (Gelfand et al. 1985, Ndamba et al. 1994), the remainder of the species (17.1%) are mixed with *Vigna unguiculata* (L.) Walp. (Table 2.2). Research conducted by Gelfand et al. (1985) and Ndamba et al. (1994) revealed that roots of *Vigna unguiculata* are usually mixed with *Eriosema englerianum* Harms, *Erythrina abyssinica* Lam. ex DC., *Euclea divinorum* Hiern, Lannea edulis (Sond.) Engl., *Terminalia sericea* Burch. ex DC. and *Vernonia amygdalina* Delile as herbal medicine for schistosomiasis.

More than three quarters (88.6%) of the plant species widely used as herbal medicines for treating and managing schistosomiasis in Zimbabwe exhibited various degrees of anthelmintic activities (Table 2.3). Research conducted by Molgaard et al. (2001) which evaluated anthelmintic activities of Zimbabwean plants traditionally used against schistosomiasis revealed that the extracts of stem and root of *Abrus precatorius*, root bark and leaves of *Ozoroa insignis* and root bark of *Ziziphus mucronata* showed the best

Table 2.2 Zimbabwean medicinal plants with potential value as schistosomiasis remedies.

Plant species	Family	Habit	Plant parts used	Other countries with similar uses	References
Abrus precatorius L.	Fabaceae	Climber	Root	DRC, South Africa	Staner and Boutique 1937, Ndamba et al. 1994, Hutchings et al. 1996
Acacia karroo Hayne	Fabaceae	Tree	Roots		Ndamba et al. 1994
Albizia antunesiana Harms	Fabaceae	Tree	Bark		Ndamba et al. 1994
Cassia abbreviata Oliver	Fabaceae	Shrub	Bark, roots		Ndamba et al. 1994
Celtis africanus N. L. Burm.	Ulmaceae	Tree	Leaves, roots		Ndamba et al. 1994
Cissampelos mucronata A. Rich.	Menispermaceae	Climber	Roots	South Africa	Gelfand et al. 1985, Hutchings et al. 1996, van Wyk and Gericke 2000, Sparg et al. 2000
Combretum imberbe Wawra	Combretaceae	Tree	Roots	Mozambique, South Africa	Gelfand et al. 1985, Venter and Venter 1996, Ribeiro et al. 2010
Dicoma anomala Sonder	Asteraceae	Herb	Roots	South Africa, Tanzania	Watt and Breyer-Brandwijk 1962, Ndamba et al. 1994
Elephantorrhiza goetzei (Harms) Harms	Fabaceae	Shrub	Roots mixed with *Piliostigma thonningii* (Schumach.) Milne-Redh.		Gelfand et al. 1985, Ndamba et al. 1994, Maroyi 2011
Eriosema englerianum Harms	Fabaceae	Herb	Roots taken orally mixed with *Vigna unguiculata* (L.) Walp.		Gelfand et al. 1985
Erythrina abyssinica Lam. ex DC.	Fabaceae	Tree	Roots taken orally mixed with *Vigna unguiculata*	Kenya	Kokwaro 1976, Gelfand et al. 1985
Euclea divinorum Hiern	Ebenaceae	Shrub	Roots taken orally mixed with *Vigna unguiculata*	South Africa, Kenya	Kokwaro 1976, Gelfand et al. 1985, Ndamba et al. 1994, Hutchings et al. 1996
Flacourtia indica (Burm. f.) Merr.	Salicaceae	Shrub	Roots	Malawi	Williamson 1975, Gelfand et al. 1985
Gymnosporia senegalensis (Lam.) Loes.	Celastraceae	Shrub	Roots	South Africa, Tanzania	Burkill 1985, Gelfand et al. 1985, Ndamba et al. 1994, Hutchings et al. 1996
Lannea discolor (Sond.) Engl.	Anacardiaceae	Tree	Bark, leaves		Ndamba et al. 1994
Lannea edulis (Sond.) Engl.	Anacardiaceae	Shrub	Roots taken orally mixed with *Vigna unguiculata*	Malawi	Gelfand et al. 1985, Ndamba et al. 1994, Chigora et al. 2007
Lecaniodiscus fraxinifolius Baker	Sapindaceae	Shrub	Bark, roots		Ndamba et al. 1994
Mondia whitei (Hook. f.) Skeels	Apocynaceae	Climber	Roots	South Africa	Gelfand et al. 1985, Hutchings et al. 1996

Table 2.2 contd. ...

...Table 2.2 contd.

Plant species	Family	Habit	Plant parts used	Other countries with similar uses	References
Ozoroa insignis Delile	Anacardiaceae	Tree	Roots		Gelfand et al. 1985, Ndamba et al. 1994
Peltophorum africanum Sonder	Fabaceae	Tree	Bark, roots		Ndamba et al. 1994
Piliostigma thonningii (Schumach.) Milne-Redh.	Fabaceae	Tree	Roots mixed with *Elephantorrhiza goetzei*		Ndamba et al. 1994, Maroyi 2011
Pterocarpus angolensis DC.	Fabaceae	Tree	Bark, roots	South Africa, Zambia	Watt and Breyer-Brandwijk 1962, Gelfand et al. 1985, Ndamba et al. 1994, Hutchings et al. 1996, Mavi 1996, Venter and Venter 1996
Ricinus communis L.	Euphorbiaceae	Shrub	Roots		Gelfand et al. 1985, Ndamba et al. 1994
Sclerocarya birrea (A. Rich.) Hochst.	Anacardiaceae	Tree	Roots	South Africa	Gelfand et al. 1985, Hutchings et al. 1996
Senna singueana (Delile) Lock	Fabaceae	Shrub	Bark, roots		Ndamba et al. 1994
Solanum delagoense Dunal	Solanaceae	Shrub	Roots		Ndamba et al. 1994
Steganotaenia araliacea Hochst	Apiaceae	Shrub	Bark, leaves, roots	Zambia	Storrs 1979, Ndamba et al. 1994
Terminalia brachystemma Welw.	Combretaceae	Shrub	Roots		Ndamba et al. 1994
Terminalia sericea Burch. ex DC.	Combretaceae	Tree	Roots taken orally mixed with *Vigna unguiculata*	Kenya, South Africa, Tanzania	Watt and Breyer-Brandwijk 1962, Kokwaro 1976, Gelfand et al. 1985, Mavi 1996
Trichilia emetica Vahl	Meliaceae	Tree	Bark, leaves		Ndamba et al. 1994
Vernonia amygdalina Delile	Asteraceae	Shrub	Roots taken orally mixed with *Vigna unguiculata*	Kenya	Kokwaro 1976, Gelfand et al. 1985, Ndamba et al. 1994
Vernonia colorata (Willd.) Drake	Asteraceae	Shrub	Roots	Ivory Coast	Burkill 1985, Gelfand et al. 1985
Vigna unguiculata (L.) Walp.	Fabaceae	Herb	Roots taken orally mixed with *Eriosema englerianum*, *Erythrina abyssinica*, *Euclea divinorum*, *Lannea edulis*, *Terminalia sericea* and *Vernonia amygdalina*		Gelfand et al. 1985, Ndamba et al. 1994
Ximenia caffra Sond.	Ximeniaceae	Shrub	Leaves, roots	South Africa	Ndamba et al. 1994, Hutchings et al. 1996, Chauke et al. 2015
Ziziphus mucronata Willd.	Rhamnaceae	Tree	Roots		Gelfand et al. 1985, Ndamba et al. 1994

Table 2.3 Zimbabwean medicinal plants used against schistosomiasis that have been screened for anthelmintic activities.

Plant species	Plant parts tested	Test organism	Concentration (mg/mL)	Safety findings	References
Abrus precatorius	Leaves, roots, stem	*Hymenolepis diminuta*	0.2–4.9	Positive - brine shrimp toxicity assay	Molgaard et al. 2001, Maregesi et al. 2016
		Schistosoma mansoni	0.6–1.5		
Acacia karroo	Leaves, roots	*Hymenolepis diminuta*	0.8–2.1	Positive - brine shrimp toxicity assay	Sparg et al. 2000, Molgaard et al. 2001, Adedapo et al. 2008, Cock and van Vuuren 2015
		Schistosoma haematobium	50.0	Positive/negative - acute and sub-acute mammalian toxicity tests	
Albizia antunesiana	Bark, leaves, roots, stem	*Hymenolepis diminuta*	1.1–6.3	Negative - MTT assay	Molgaard et al. 2001, Chipiti et al. 2013
Cassia abbreviata	Bark, leaves, roots	*Hymenolepis diminuta*	0.5–67.5	Positive - brine shrimp toxicity assay	Molgaard et al. 2001, Moshi et al. 2007
Celtis africanus	Leaves, roots	*Hymenolepis diminuta*	0.5–3.9	Negative - ames and VITOTOX® and positive - micronucleus test	Molgaard et al. 2001, Elgorashi et al. 2003, Taylor et al. 2003
Combretum imberbe	Leaves, roots	*Schistosoma haematobium*	12.5	Negative - biochemical induction assay	McGaw et al. 2001, Sparg et al. 2001
Dicoma anomala	Roots	*Hymenolepis diminuta*	1.0	Negative - brine shrimp toxicity assay	Molgaard et al. 2001, Munodawafa et al. 2017
Elephantorrhiza goetzei	Bark, fruits, leaves, roots, stem	*Hymenolepis diminuta*	0.5–42.2	Positive - brine shrimp toxicity assay	Molgaard et al. 2001, Wanjala and Majinda 2001
	Bark	*Schistosoma mansoni*	0.8		
Erythrina abyssinica	Bark, leaves, stem	*Ascaridia galli*	0.8–1.7	Positive - *in vivo* haematinic activity	Lagu and Kayanja 2013, Musyoka et al. 2016
Euclea divinorum	Bark	*Caenorhabditis elegans*	1.0	Positive (toxic) - ames and VITOTOX®	McGaw et al. 2000, Sparg et al. 2000, Elgorashi et al. 2003
	Root	*Schistosoma haematobium*	25.0		
Gymnosporia senegalensis	Bark, leaves, roots, stem	*Hymenolepis diminuta*	2.5–6.3	Positive - vero cells	Molgaard et al. 2001, Nabende et al. 2015
	Bark	*Schistosoma mansoni*	100		
Lannea discolor	Leaves, stem	*Hymenolepis diminuta*	2.5	Positive - MTT assay and vero African monkey kidney cells	Molgaard et al. 2001, Kabongo-Kayoka et al. 2016
Lannea edulis	Leaves, stem	*Hymenolepis diminuta*	3	Negative - brine shrimp toxicity assay	Molgaard et al. 2001, Munodawafa et al. 2017
Lecaniodiscus fraxinifolius	Leaves, roots, stem	*Hymenolepis diminuta*	1.6–3.1	-	Molgaard et al. 2001
Mondia whitei	Roots	*Schistosoma haematobium*	50	Positive - acute and sub-acute mammalian toxicity tests	Sparg et al. 2000, Joseph et al. 2015
Ozoroa insignis	Bark, leaves, roots, stem	*Hymenolepis diminuta*	0.8–51.3	Positive - brine shrimp toxicity assay	Molgaard et al. 2001, Haule et al. 2012
	Bark, leaves, roots	*Schistosoma mansoni*	25.3–33.8		

Table 2.3 contd. ...

...Table 2.3 contd.

Plant species	Plant parts tested	Test organism	Concentration (mg/mL)	Safety findings	References
Peltophorum africanum	Bark, leaves, roots, stem	*Hymenolepis diminuta*	0.5–0.8	Positive - vero cells	Molgaard et al. 2001, Bizimenyera et al. 2006b, Bizimenyera 2007, Samie et al. 2009
	Bark, leaves, roots	*Hymenolepis contortus*	1.0–5.0	Negative - brine shrimp toxicity assay; and vero monkey kidney cells cytotoxicity assay	
Piliostigma thonningii	Bark, leaves, roots, stem	*Hymenolepis diminuta*	0.3–2.2	Positive - acute and sub-acute mammalian toxicity tests	Molgaard et al. 2001, Ukwuani et al. 2012
Pterocarpus angolensis	Bark, leaves, stem	*Hymenolepis diminuta*	12.8–67.6	Positive - brine shrimp toxicity assay	Molgaard et al. 2001, McGaw et al. 2007
	Bark	*Schistosoma mansoni*	70.0		
Ricinus communis	Leaves	*Caenorhabditis elegans*	1.0	Negative - ames and VITOTOX®; and positive - brine shrimp toxicity assay	Sparg et al. 2000, Elgorashi et al. 2003, Taylor et al. 2003, McGaw et al. 2007
	Leaves + stem				
	Roots	*Schistosoma haematobium*	25.0	Positive - micronucleus test	
Sclerocarya birrea	Bark	*Caenorhabditis elegans*	0.5–2.0	Negative - brine shrimp toxicity assay	McGaw et al. 2007
Senna singueana	Bark, leaves, roots, stem	*Hymenolepis diminuta*	0.5–4.0	Negative - modified Lorke's method	Molgaard et al. 2001, Ior et al. 2015
Solanum delagoense	Leaves, roots	*Hymenolepis diminuta*	0.4–4.2	-	Molgaard et al. 2001
Steganotaenia araliacea	Bark, leaves, roots, stem	*Hymenolepis diminuta*	7.8–33.8	Positive - acute and sub-acute mammalian toxicity tests	Molgaard et al. 2001, Agunu et al. 2003
Terminalia brachystemma	Fruits, leaves, roots	*Hymenolepis diminuta*	0.5–1.7	-	Molgaard et al. 2001
Trichilia emetica	Bark, leaves, roots	*Hymenolepis diminuta*	21.2–25.6	Negative - ames and VITOTOX®; positive - micronucleus test	Molgaard et al. 2001, Elgorashi et al. 2003, Taylor et al. 2003
Vernonia amygdalina	Bark, leaves, roots, stem	*Hymenolepis diminuta*	3.4–11.6	Negative - acute and sub-acute mammalian toxicity tests	Molgaard et al. 2001, Amole et al. 2006
Vernonia colorata	Roots	*Schistosoma haematobium*	50.0	Negative - ames and VITOTOX®; and positive (toxic) - micronucleus test and comet assay	Sparg et al. 2000, Elgorashi et al. 2003, Taylor et al. 2003
Ximenia caffra	Bark, leaves, roots, stem	*Hymenolepis diminuta*	0.5–0.8	Positive - vero cells	Kamuhabwa et al. 2000, Sparg et al. 2000, Molgaard et al. 2001, Samie et al. 2009
	Roots	*Schistosoma haematobium*	50.0	Positive - antiproliferative assay with three human cell lines (HeLa, HT29 and A431)	
Ziziphus mucronata	Bark, leaves, roots, stem	*Hymenolepis diminuta*	0.02–51.8	Positive - ames and VITOTOX®; micronucleus test and comet assay; and brine shrimp toxicity assay	Sparg et al. 2000, Molgaard et al. 2001, Elgorashi et al. 2003, Taylor et al. 2003, McGaw et al. 2007
	Roots	*Schistosoma haematobium*	12.5		

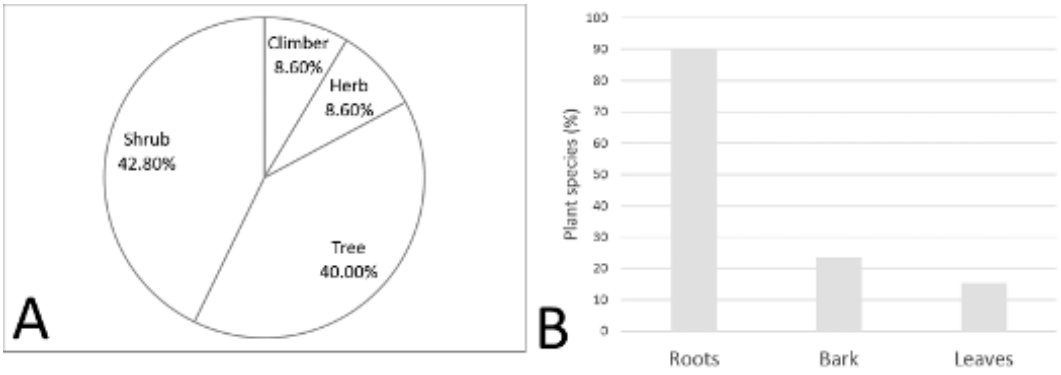


Fig. 2.1 Characteristics of the plants used as herbal medicines in treating and managing schistosomiasis in Zimbabwe. (A) Growth form habit represented in pie diagram and (B) plant parts used represented in bar chart.

results against tapeworms. According to Molgaard et al. (2001), the best results against schistosomules were obtained with stem and root extracts of *Abrus precatorius* and stem bark of *Elephantorrhiza goetzei*. Similarly, Ndamba et al. (1994) investigated herbal medicines used against schistosomiasis in Zimbabwe based on interviews with 286 traditional healers and the authors documented a total of 47 plant species. Based on this survey, the seven most commonly used plant species *Abrus precatorius*, *Ozoroa insignis*, *Dicoma anomala*, *Ximenia caffra*, *Lannea edulis*, *Elephantorrhiza goetzei* and *Pterocarpus angolensis* were collected, prepared as described by the traditional healers, their efficacy was evaluated using laboratory animals previously exposed to *Schistosoma haematobium* cercariae. The anthelmintic activity from the extract of the bark of Pterocarpus angolensis was found to be comparable to that of PZQ, the root bark of *Ozoroa insignis* and the root of *Abrus precatorius* were also lethal to adult schistosomes (Ndamba et al. 1994).

Future research and conclusion

From a research and development point of view, many herbal medicines used against schistosome infections have not received any major emphasis from government departments, non-governmental organisations in Zimbabwe, and as such, plant species used against schistosomiasis have remained underutilized. Nevertheless, the majority of the medicinal plants documented in this study achieve their efficacies by reducing *Schistosoma* species egg-hatching and larval motility or metabolism (Ndamba et al. 1994, Sparg et al. 2000, Mølgaard et al. 2001). Unfortunately, the modes of action of these medicinal plants have not been fully explored. Therefore, contemporary research involving herbal medicines used against schistosomiasis is promising, the results obtained so far are too preliminary and sometimes too general to be used to explain and support usage of such species against schistosome infections. In addition to this, most of the anthelmintic evaluations done so far, are routine screenings using standard procedures lacking molecular mechanisms of the pharmacological effects of the herbal medicines. There is not yet enough systematic data regarding the pharmacokinetics and clinical research on medicinal species used against schistosome infections. There are also very few experimental animal studies, randomized clinical trials and target-organ toxicity studies involving some of these herbal medicines and their derivatives that have been carried out so far. Therefore, future studies should identify the bioactive components, details of the molecular modes or mechanisms of action, pharmacokinetics and physiological pathways for specific bioactives of the documented plant species that are used against schistosome infections.

Acknowledgement

The author would like to express his gratitude to the National Research Foundation and Govan Mbeki Research and Development Centre, University of Fort Hare for financial support to conduct this research.

References

Adenowo, A.F., Oyinloye, B.E., Ogunyinka, B.I. and Kappo, A.P. 2015. Impact of human schistosomiasis in sub-Saharan Africa. Braz. J. Infect. Dis. 19: 196–205.

Adedapo, A.A., Sofidiya, M.O., Masika, P.J. and Afolayan, A.J. 2008. Safety evaluations of the aqueous extract of *Acacia karroo* stem bark in rats and mice. Rec. Nat. Prod. 2: 128–134.

Agunu, A., Ibrahim, N.D.G., Onyiloyi, G.A. and Abdurahman, E.M. 2003. Toxicity of stem-bark extract of *Steganotaenia araliacea* in rats. Niger. J. Nat. Prod. Med. 7: 65–67.

Amole, O.O., Izegbu, M.C., Onakoya, J.A.A. and Dada, M.O. 2006. Toxicity studies of the aqueous extract of *Vernonia amygdalina*. Biomed. Res. 17: 39–40.

Aragon, A.D., Imani, R.A., Blackburn, V.R., Cupit, P.M., Melman, S.D., Goronga, T., Webb, T., Loker, E.S. and Cunningham, C. 2009. Towards an understanding of the mechanism of action of praziquantel. Mol. Biochem. Parasit. 164: 57–65.

Aremu, A.O., Finnie, J.F. and van Staden, J. 2012. Potential of South African medicinal plants used as anthelmintics: their efficacy, safety concerns and reappraisal of current screening methods. S. Afr. J. Bot. 82: 134–150.

Bizimenyera, E.S. 2007. The potential role of antibacterial, antioxidant and antiparasitic activity of *Peltophorum africanum* Sond. (Fabaceae) extracts in ethnoveterinary. PhD Thesis, University of Pretoria, Pretoria.

Bizimenyera, E.S., Githiori, J.B., Swan, G.E. and Eloff, J.N. 2006. *In vitro* ovicidal and larvicidal activity of the leaf, bark and root extracts of *Peltophorum africanum* Sond. (Fabaceae) on *Haemonchus contortus*. J. Anim. Vet. Adv. 5: 608–614.

Burkill, H.M. 1985. The useful plants of West Tropical Africa. Royal Botanic Gardens, Kew, London.

Chauke, M.A., Shai, L.J., Mogale, M.A., Tshisikhawe, M.P. and Mokgotho, M.P. 2015. Medicinal plant use of villagers in the Mopani district, Limpopo province, South Africa. Afr. J. Tradit. Complem. 12: 9–26.

Chimbari, M.J. 2012. Enhancing schistosomiasis control strategy for Zimbabwe: Building on past experiences. J. Parasit. Res. Volume 2012, Article ID 353768.

Chigora, P., Masocha, R. and Mutenheri, F. 2007. The role of indigenous medicinal knowledge (IMK) in the treatment of ailments in rural Zimbabwe: the case of Mutirikwi communal lands. J. Sustain. Dev. in Africa 9: 26–43.

Chipiti, T., Ibrahim, M.A., Koorbanally, N.A. and Islam, S. 2013. *In vitro* antioxidant activities of leaf and root extracts of *Albizia antunesiana* Harms. Acta Pol. Pharm. 70: 1035–1043.

Clark, T.E., Appleton, C.C. and Kvalsvig, J.D. 1997. Schistosomiasis and the use of indigenous plant molluscicides: a rural South African perspective. Acta Trop. 66: 93–107.

Cock, I.E. and van Vuuren, S.F. 2015. South African food and medicinal plant extracts as potential antimicrobial food agents. J. Food Sci. Tech. 52: 6879–6899.

Couto, F.F.B., Coelho, P.M.Z., Araújo, N,, Kusel, J.R., Katz, N., Jannotti-Passos, L.K. and Mattos, A.C.A. 2011. *Schistosoma mansoni*: a method for inducing resistance to praziquantel using infected Biomphalaria glabrata snails. Mem. I. Oswaldo Cruz 106: 153–157.

Doenhoff, M.J., Cioli, D. and Utzinger, J. 2008. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Current Opinion Infectious Diseases 21: 659–667.

Elgorashi, E.E., Taylor, J.L.S., Maes, A., van Staden, J., De Kimpe, N. and Verschaeve, L. 2003. Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicol. Lett. 143: 195–207.

Fallon, P.G., Sturrock, R.F., Niang, A.C. and Doenhoff, M.J. 1995. Short report: diminished susceptibility to praziquantel in a Senegal isolate of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. 53: 61–62.

Fallon, P.G. and Doenhoff, M.J. 1994. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. Am. J. Trop. Med. Hyg. 51: 83–88.

Gelfand, M., Drummond, R.B., Mavi, S. and Ndemera, B. 1985. The traditional medical practitioner in Zimbabwe: his principles of practice and pharmacopoeia. Mambo Press, Gweru.

Gryseels, B. and de Vlas, S.J. 1996. Worm burdens in schistosome infections. Parasitol. Today 12: 115–119.

Haule, E.E., Moshi, M.J., Nondo, R.S.O., Mwangomo, D.T. and Mahunnah, R.L.A. 2012. A study of antimicrobial activity, acute toxicity and cytoprotective effect of a polyherbal extract in a rat ethanol-HCl gastric ulcer model. BMC Res. Notes 5: 546.

Hedberg, I. and Staugård, F. 1989. Traditional medicinal plants: traditional medicine in Botswana. Ipeleng Publishers, Gaborone.

Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A. 1996. Zulu medicinal plants. University of Natal Press, Natal.

Inobaya, M.T., Olveda, R.M., Chau, T.N.P., Olveda, D.U. and Ross, A.G.P. 2014. Prevention and control of schistosomiasis: a current perspective. Res. Rep. Trop. Med. 5: 65–75.

Ior, L.D., Wannang, N.N., Ior, C.A. and Amagon, K.I. 2015. *In vivo* assessment of the antimalarial activity of *Cassia singueana* and *Cymbopogon citrutus*. Der Pharma Chemica 7: 272–278.

Ismail, M., Metwally, A., Farghaly, A., Bruce, J., Tao, L.F. and Bennett, J.L. 1996. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. Am. J. Trop. Med. Hyg. 55: 214–218.

Ismail, M.M., Taha, S.A., Farghaly, A.M. and El-Azony, A.S. 1994. Laboratory induced resistance to praziquantel in experimental schistosomiasis. J. Egypt. Soc. Parasitol. 24: 685–695.

Joseph, O., Kihdze, T.J., Katusiime, B., Imanirampa, L., Waako, P., Bajunirwe, F. and Ganafa, A.A. 2015. Toxicity of four herbs used in erectile dysfunction, *Mondia whiteii*, *Cola acuminata*, *Urtica massaica* and *Tarenna graveolens* in male rats. Afr. J. Pharm. Pharmaco. 9: 756–763.

Kabongo-Kayoka, P.N., Eloff, J.N., Obi, C.L. and McGaw, L.J. 2016. Antimycobacterial activity and low cytotoxicity of leaf extracts of some African Anacardiaceae tree species. Phytother. Res. 30: 2001–2011.

Kamuhabwa, A., Nshimo, C. and DeWitte, P. 2000. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. J. Ethnopharmacol. 70: 143–149.

King, C.H. 2006. Long-term outcomes of school-based treatment for control of urinary schistosomiasis: a review of experience in Coast province, Kenya. Mem. I. Oswaldo Cruz 101(Suppl): 299–306.

Kokwaro, J.O. 1976. Medicinal plants of East Africa. East African Literature Bureau, Nairobi.

Lagu, C. and Kayanja, F.B.I. 2013. The *in vitro* antihelminthic efficacy of *Erythrina abyssinica* extracts on *Ascaridia galli*. pp. 269–281. *In*: Carreira, R.P. (ed.). Insights from Veterinary Medicine, InTech, Rejeka.

Magaisa, K., Taylor, M., Kjetland, E.F. and Naidoo, P.J. 2015. A review of the control of schistosomiasis in South Africa. S. Afr. J. Sci. 111(11/12), Art. #2014–0427.

Magnussen, P. 2003. Treatment and re-treatment strategies for schistosomiasis control in different epidemiological settings: a review of 10 years' experiences. Acta Trop. 86: 243–254.

Maregesi, S.M., Mwakigonja, A.R. and Urio, P. 2016. Toxicity evaluation of *Abrus precatorius* seeds collected from Bunda district, Tanzania. Schol. Acad. J. Pharm. 5: 399–405.

Maroyi, A. 2011. Ethnobotanical study of medicinal plants used by people in Nhema communal area, Zimbabwe. J. Ethnopharmacol. 136: 347–354.

Mavi, S. 1996. Medicinal plants and their uses in Zimbabwe. pp. 67–73. *In*: Norman, H., Snyman, I. and Cohen, M. (eds.). Indigenous knowledge and its uses in Southern Africa. Human Sciences Research Council, Pretoria.

McGaw, L.J., Jäger, A.K. and van Staden, J. 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J. Ethnopharmacol. 72: 247–263.

McGaw, L.J., Rabe, T., Sparg, S.G., Jäger, A.K., Eloff, J.N. and van Staden, J. 2001. An investigation on the biological activity of *Combretum* species. J. Ethnopharmacol. 75: 45–50.

McGaw, L.J., van der Merwe, D. and Eloff, J.N. 2007. *In vitro* anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine. Vet. J. 173: 366–372.

Melman, S.D., Steinauer, M.L., Cunningham, C., Kubatko, L.S., Mwangi, I.N., Wynn, N.B., Mutuku, M.W., Karanja, D.M.S., Colley, D.G., Black, C.L., Secor, W.E., Mkoji, G.M. and Loker, E.S. 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. PLOS Neglect. Trop. D. 3: e504.

Midzi, N., Sangweme, D., Zinyowera, S., Mapingure, M.P., Brouwer, K.C., Munatsi, A., Mutapi, F., Mudzori, J., Kumar, N., Woelk, G. and Mduluza, T. 2008. The burden of polyparasitism among primary schoolchildren in rural and farming areas in Zimbabwe. T. Roy. Soc. Trop. Med. H. 102: 1039–1045.

Midzi, N., Mduluza, T., Chimbari, M.J., Tshuma, C., Charimari, L., Mhlanga, G., Manangazira, P., Munyati, S.M., Phiri, I., Mutambu, S.L., Midzi, S.S., Ncube, A., Muranzi, L.P., Rusakaniko, S. and Mutapi, F. 2014. Distribution of schistosomiasis and soil transmitted helminthiasis in Zimbabwe: towards a national plan of action for control and elimination. PLOS Neglect. Trop. D. 8: e3014.

Moshi, M.J., van der Beukel, C.J.P., Hamza, O.J.M., Mbwambo, Z.H., Nondo, R.O.S., Masimba, P.J., Matee, M.I.N., Kapingu, M.C., Mikx, F., Verweij, P.E. and van der Ven, A.J.AM. 2007. Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. Afr. J. Tradit. Complem. 4: 219–225.

Mølgaard, P., Nielsen, S.B., Rasmussen, D.E., Drummond, R.B., Makaza, N. and Andreassen, J. 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. J. Ethnopharmacol. 74: 257–264.

Munodawafa, T., Moyo, S., Chipurura, B. and Chagonda, L. 2017. Brine shrimp lethality bioassay of some selected Zimbabwean traditional medicinal plants. Int. J. Phytopharmacol. 8: 8–12.

Musyoka, T.M., Dorothy, N.W., Wycliffe, A.M., Juma, K.K., Nzioka, M.D., Maina, D., Waithaka, S.K., Ngugi, M.P., Orinda, G.O., Karau, G.M. and Njagi, E.N.M. 2016. *In vivo* antianaemic effect and safety of aqueous extracts of *Erythrina abyssinica* and *Zanthoxylum usambarensis* in mice models. J. Hematol. Thromb. Dis. 4: 242.

Mutapi, F. 2015. Changing policy and practice in the control of pediatric schistosomiasis. Pediatrics 135: 1–9.

Mutapi, F., Rujeni, N., Bourke, C., Mitchell, K., Appleby, L., Nausch, N., Midzi, N. and Mduluza, T. 2011. *Schistosoma haematobium* treatment in 1–5 year old children: safety and efficacy of the antihelminthic drug praziquantel. PLOS Neglect. Trop. D. 5: e1143.

Nabende, P.N., Karanja, S.M., Mwatha, J.K. and Wachira, S.W. 2015. Anti-proliferative activity of *Prunus africana*, *Warburgia stuhlmannii* and *Maytenus senegalensis* extracts in breast and colon cancer cell lines. Eur. J. Med. Plant. 5: 366–376.

Ndamba, J., Nyazema, N., Makaza, N., Anderson, C. and Kaondera, K.C. 1994. Traditional herbal remedies used for the treatment of urinary schistosomiasis in Zimbabwe. J. Ethnopharmacol. 42: 125–132.

Ndjonka, D., Rapado, L.N., Silber, A.M., Liebau, E. and Wrenger, C. 2013. Natural products as a source for treating neglected parasitic diseases. Int. J. Mol. Sci. 14: 3395–3439.

Neves, B.J., Andrade, C.H. and Cravo, P.V.L. 2015. Natural products as leads in schistosome drug discovery. Molecules 20: 1872–1903.

Ojewole, O.J.A. 2004. Indigenous plants and schistosomiasis control in south africa: molluscicidal activity of some Zulu medicinal plants. B. Latinoam. Caribe Pl. 3: 8–22.

Pedersen, U.B., Karagiannis-Voules, D.-A., Midzi, N., Mduluza, T., Mukaratirwa, S., Fensholt, R., Vennervald, B.J., Kristensen, T.K., Vounatsou, P. and Stensgaard, A.-S. 2017. Comparison of the spatial patterns of schistosomiasis in Zimbabwe at two points in time, spaced twenty-nine years apart: is climate variability of importance? Geospatial Health 12: 505.

Ribeiro, A., Romeiras, M.M., Tavares, J. and Faria, M.T. 2010. Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. J. Ethnobiol. Ethnomed. 6: 33.

Ross, A.G., Chau, T.N., Inobaya, M.T., Olveda, R.M., Li, Y. and Harn, D.A. 2017. A new global strategy for the elimination of schistosomiasis. Int. J. Infect. Dis. 54: 130–137.

Samie, A., Obi, C.L., Lall, N. and Meyer, J.J.M. 2009. *In vitro* cytotoxicity and antimicrobial activities, against clinical isolates of *Campylobacter* species and *Entamoeba histolitica*, of local medicinal plants from Venda region, in South Africa. Ann. Trop. Med. Parasit. 103: 159–170.

Sparg, S.G., van Staden, J. and Jäger, A.K. 2000. Efficiency of traditionally used South African plants against schistosomiasis. J. Ethnopharmacol. 72: 1–6.

Staner, P. and Boutique, R. 1937. Matériaux pour l' étude des plantes médicinales indigènes du Congo Belge. Académie Royale de Belgique, Bruxelles.

Steinmann, P., Keiser, J., Bos, R., Tanner, M. and Utzinger, J. 2006. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect. Dis. 6: 411–425.

Storrs, A.E.G. 1979. Know your trees: some of the most common trees found in Zambia. The Forest Department, Ndola.

Taylor, J.L.S., Elgorashi, E.E., Maes, A., van Gorp, U., De Kimpe, N., van Staden, J. and Verschaeve, L. 2003. Investigating the safety of plants used in South African traditional medicine: testing for genotoxicity in the micronucleus and alkaline comet assays. Environ. Mol. Mutag. 42: 144–154.

Ukwuani, A.N., Ihebunna, O., Samuel, R.M. and Peni, I.J. 2012. Acute oral toxicity and antiulcer activity of *Piliostigma thonningii* leaf fraction in Albino rats. B. Environ. Pharmacol. Life Sci. 2: 41–45.

Van Wyk, B.-E. and Gericke, N. 2000. People's plants: a guide to useful plants of southern Africa. Briza Publications, Pretoria.

Venter F and Venter J. 1996. Making the most of indigenous trees. Briza Publications, Pretoria.

Wanjala, C.C.W. and Majinda, R.R.T. 2001. A new stilbene glycoside from *Elephantorrhiza goetzei*. Fitoterapia 72: 649–655.

Watt, J.M. and Breyer-Bradwijk, M.G. 1962. The medicinal and poisonous plants of southern and eastern Africa. E. & S. Livingstone, Edinburgh.

Williamson, J. 1975. Useful plants of Malawi. University of Malawi, Zomba.

World Health Organization (WHO). 2017a. Schistosomiasis fact sheet. World Health Organization Media Centre, Geneva. Available from: http://www.who.int/mediacentre/factsheets/fs115/en/, accessed on 29 July 2017.

World Health Organization (WHOb). 2017. Schistosomiasis: epidemiological situation. World Health Organization Media Centre, Geneva. Available from: http://www.who.int/schistosomiasis/epidemiology/en/, accessed on 29 July 2017.

3

Ethnobotany: Medicinal Plants Used in the Management of Hypertension

Patricia Landazuri,[1,*] *Nelsy Loango Chamorro*[2] and *Beatriz Restrepo Cortes*[1]

Introduction

Hypertension is a worldwide disease, and it is the most common serious chronic health problem and also a high risk factor for myocardial infarction, arteriosclerosis, stroke, and end-stage renal disease. Twenty five percent of the world's adult population has hypertension and this is likely to increase by 30% by 2025 (WHO 2013, World Heart Federation 2015).

The primary treatment for hypertension is: stress management, maintaining proper weight, reducing salt intake, limiting alcohol consumption, aerobic physical activity and dietary control. The hypertension guidelines indicated that when these treatments are not enough, drugs should be administered (Mancia et al. 2013, James et al. 2014); antihypertensive drugs have proved to be effective, but they have many side effects, such as reduced renal function, dry cough, angioedema among others effects (Kiriyama et al. 2016). Hence, the management of hypertension by herbal medicine can be a complementary treatment (Landázuri et al. 2017, Leung et al. 2017), and ethnobotanical studies for use of medicinal plants for the management of hypertension can be of great help (Baharvand-Ahmadi et al. 2016, Rawat et al. 2016).

Ethnobotany is a specific field of scientific study of the plants, and also their relationship with people. Modern ethnobotanists strive to collect all available data on the use of plants, to document the biodiversity of medicinal plants and the methods of use (Popovi et al. 2016).

Blood pressure (BP) is controlled by several mechanism: local mechanisms of BP control (i.e., nitric oxide (NO), endothelin), neural mechanisms (i.e., sympathetic nervous system), renal-endocrine mechanisms (i.e., Renin-Angiotensin-Aldosterone-System (RAAS), and a variety of other hormones (i.e., antidiuretic hormone)).

RAAS activation is a very important mechanism responsible for regulation of BP and the Angiotensin Converting Enzyme (ACE), is the key enzyme that regulates RAAS, which is a system that is also involved in the regulation of plasma sodium concentration.

Bioactive compounds in plants, such as polyphenols and peptides, may be useful in the prevention of cardiovascular diseases, such as hypertension (Medina-Remón et al. 2015, Shayganni et al. 2016, Ferreira et al. 2017).

[1] Faculty of Health Sciences. Medicine, and Biomedical Science programs, Universidad Del Quindío, Armenia-Quindío, Colombia.
[2] Faculty of Basic Science and Technologies, Biology carrer program, Universidad Del Quindío, Armenia-Quindío, Colombia.
* Corresponding author: plandazu@uniquindío.edu.co

Bioactive compounds in plants including polyphenols and peptides, may be useful in the prevention of cardiovascular diseases, such as hypertension; thus, the antihypertensive activity of several of these metabolites result from inhibition of ACE, or calcium channel blockade, among other mechanisms of action (Rawat et al. 2016). Thus in recent years, the inhibitory effect on ACE activity of *Passiflora edulis, Phthirusa pyrifolia*, and *Eucommia ulmoides* Oliver has been demonstrated (Restrepo et al. 2013, Restrepo et al. 2014, Yan et al. 2017) and calcium channel blockade of *Achillea wilhelmsii* (Niazmand et al. 2014, Rawat et al. 2016).

Thus, the objective of this chapter is to contribute to the review of some medicinal plants, whose extracts or active compounds have demonstrated efficacy on blood pressure regulation systems, such as the RAAS, the blocking of calcium channels, nitric oxide release and arterial vasorelaxation.

Ethnobotanical studies and hypertension

Ethnobotanical studies can be defined as the scientific research of plants, as they are used in native cultures for food, medicine, spiritual therapies, rituals, pesticides or other applications, used alone or in combination to diagnose, treat, and prevent diseases or maintain well-being (Olowokudejo et al. 2008).

Ethnobotanical studies have provided reliable guides for use of herbal medicines that nature offers; nature is the greatest source of medicaments for many health problems, consequently, today herbal medicines are used alone or in combination with traditional pharmaceutical compounds to treat various ailments (Falzon et al. 2017, Lin et al. 2017), without forgetting that a high percentage of the new medicaments approved, have been derived directly or indirectly from natural products (Dutra et al. 2016, Gerwick 2017, Petroni et al. 2017); in these ethnobotanical studies, there are many medicinal plants recommended by native communities for the treatment of hypertension; this general knowledge provides new areas of research related to the active principles and mechanisms of action of these plants in hypertension management (Ahmad et al. 2015, Baharvand-Ahmadi et al. 2016, Lee and Hur 2017).

Hypertension

Blood pressure is the force exerted by circulating blood on the artery walls, which originates in the pumping action of the heart, and is produced primarily by the contraction of the heart (James et al. 2014). BP is measured in millimeters of mercury (mm Hg). It is recorded as two indexes: Systolic Blood Pressure (SBP), which shows the force that blood exerts against artery walls when the heart beats to pump blood to the peripheral organs and tissues, and Diastolic Blood Pressure (DBP), indicates the force that the blood is exerting against artery walls while the heart is resting between beats. It is postulated that the normal values of systolic pressure for healthy individuals should vary between 100 mm Hg and 140 mm Hg and the diastolic pressure values varies between 60 mm Hg and 100 mm Hg (Mancia et al. 2013, WHO 2013, James et al. 2014, Vardanyan et al. 2016).

High blood pressure or arterial hypertension is a serious medical condition. Hypertension has been called the "silent killer", because people with hypertension do not have any signs or symptoms, and many do not even know they have it. According to the American or European Societies of Hypertension a person suffers this condition when its SBP is consistently higher than 140 mm Hg and/or its DBP is $\geq$ 90 mm Hg (Mancia et al. 2013, James et al. 2014).

For 90 to 95% of patients, the causes of hypertension are unknown, this hypertension is classified as essential or primary hypertension. The remaining 5 to 10% are cases of secondary hypertension caused by underlying heart, kidney, or endocrinal diseases, certain cancers, or use of cocaine, amphetamines, thyroid supplements, or corticosteroids (Mancia et al. 2013, James et al. 2014).

WHO estimates that approximately one billion people all over the world suffer from hypertension. It is further estimated that this number will escalate to more than 1.56 billion by the year 2025 (WHO 2013) and that hypertension occurrence is around 20–30% in the adult population in developed countries (Chockalingam et al. 2006, WHO 2013, Ahmad et al. 2015, World Heart Federation 2015).

On the other hand, in a large number of studies the relationships between high BP values and fatal cardiovascular and renal events have been addressed (Nangia et al. 2016, Textor 2017, Torlasco et al. 2017).

WHO also estimates that hypertension is responsible for at least 45% of deaths due to heart diseases, and that 51% of deaths worldwide are due to hypertension, which is one of the most important causes (WHO 2013, Lima Prando et al. 2015).

Many antihypertensive agents, such as diuretics, β blockers, calcium-channel blockers, and RAAS blockers as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers among others, are used separately or in combination to treat this disease (Charlton and Thompson 2015, Vardanyan et al. 2016). However and despite its effectiveness, these medications can cause serious side effects, like adverse cardiovascular outcomes, diabetes, dry coughing, bradycardia, arrhythmia, electrolyte disturbances, fluid retention and headache among others (Ahmed et al. 2015, Charlton and Thompson 2015, Nishioka et al. 2015, Kiriyama et al. 2016). Also non adherence to antihypertensive treatment is a critical contributor to suboptimal blood pressure control (Alsabbagh et al. 2014).

These problems have motivated researchers to find new medicines from medicinal plants, to control hypertension and with fewer side effects (Nunes et al. 2015, Baharvand-Ahmadi et al. 2016, Popovi et al. 2016). Recently, several ethnobotanical studies showed that hundreds of plants are used worldwide for empirical hypertension treatment (Ahmad et al. 2015, Nunes et al. 2015, Baharvand-Ahmadi et al. 2016, Lee et al. 2017). On the other hand, the results of several ethnobotanical surveys indicated that interviewed patients used medicinal plants to treat hypertension because phytotherapy is cheaper, more efficient and better than modern medicine (Charlton and Thomson 2015, Eddouks et al. 2017).

In this context, the treatment adherence, the cost-benefit ratio, cultural beliefs and well-being, achieved by the use of medicinal plants for the hypertension treatment, generate the idea of reliability and safety of these herbal medicines, contributing to improve/increase the therapeutic repository tools and helping adherence to antihypertensive medications (Lima Prando et al. 2015).

Blood pressure control by medicinal plants

Various mechanisms have been suggested for the maintenance of blood pressure in humans. One such well known system is the RAAS, another mechanisms for reduction of hypertension are blocking Ca^{2+} channels in cellular membrane (by calcium antagonists), or blocking, the sympathetic and parasympathetic nervous system, because some blood vessels are innervated by sympathetic adrenergic nerves, which release norepinephrine (NE) as a neurotransmitter. Other blood vessels are innervated by parasympathetic or sympathetic cholinergic nerves, both which release acetylcholine as their primary neurotransmitter. NE binds alpha-1 and alpha-2 adrenoceptors to cause smooth muscle contraction and vasoconstriction; while acetylcholine binds to muscarinic receptors on the smooth muscle and/or endothelium and results in vasodilation.

On the other hand, ACE inhibition is largely related to two types of blood pressure control, RAAS and Nitric Oxide System (NOS). In the first control, ACE cleaves the C-terminal dipeptide His-Leu of Angiotensin I (ANG I), resulting in Angiotensin II (ANG II), that increases blood pressure through binding to receptors AT1 and AT2, especially, AT1 receptor to induce vasoconstriction (Patel et al. 2017). ACE is also the main enzyme that destroys bradykinin which binds to beta-2-receptors that cause an increase in intracellular Ca^{2+} level. The increased Ca^{2+} level and bradykinin lead to nitric oxide synthase to convert L-arginine to nitric oxide, the second system, which is a potent vasodilator (Balakumar and Jugadeesh 2014).

Ethnobotanical studies have described hundreds of plants used by communities for hypertension management, 99 plants were found in only four papers (2015–2016), Baharvand-Ahmadi et al. (2016) reported 27 medicinal plants from 22 families, Polat et al. (2015) found five plants from three families, Ahmad et al. (2015) reported 46 plants from 29 families and Rawat et al. (2016), described 21 plants. In the last report they described the action mode of the plants and their clinical evidences.

In this chapter we included 10 plants, which were chosen because they were cited three or more times in the reviewed literature and have described compounds and a possible mechanism of action.

Achillea wilhelmsii C. Koch. (*A. wilhelmsii*), Asteraceae family. Common name yarrow. This plant contains flavonoids and sesquiterpene lactones, which have shown to be effective in lowering hypertension in men and women (Asgary et al. 2000). They showed that hypertensive subjects treated with hydroalcoholic extract (15–20 drops) twice daily for more than 6 months, had decreased diastolic and systolic blood pressure after 2 and 6 months, respectively ($p < 0.05$).

Allium sativum L. (*A. sativum*), Liliaceae family. Common name: garlic. Several epidemiological studies suggest an antihypertensive effect of *A. sativum* and its bioactive components, principally S-allyl cysteine and allicin (Ried et al. 2014). These authors proposed that garlic-derived polysulfides stimulate the production of the vascular gaso-transmitter hydrogen sulfide (H_2S) and enhance the regulation of endothelial Nitric Oxide (NO), which induce smooth muscle cell relaxation, vasodilation, and blood pressure reduction. On the other hand, Ashraf et al. (2013), evaluated the effects of garlic on blood pressure in patients with essential hypertension; patients received between 300 and 1500 mg in divided doses per day for 24 weeks; the study showed significant decrease for both systolic and diastolic blood pressure in a dose dependent manner.

Annona muricata L. (*A. muricata*), Annonaceae family. Common name: soursop graviola or guanabana. The tree grows natively in the Caribbean and Central America; more than 200 compounds have been isolated and identified from different parts of this plant, phenols, alkaloids and acetogenins were the most important and effective compounds isolated from leaves, barks, seeds, roots and fruits (Patel and Patel 2016). The leaf extract of the plant (9.17–48.5 mg/kg body weight) administered to normotensive Sprague-Dawley rats has been reported to lower an elevated blood pressure through peripheral mechanisms involving antagonism of Ca^{+2} (Nwokocha et al. 2012).

Artemisia campestris L. (*A. campestris*), Asteraceae family. *C*ommon names: field sagewort, beach wormwood, field sagebrush, field wormwood; it is a medicinal herb traditionally used to treat hypertension. The oil of the air-dried (AcEO) plant has the spathulenol (10.19%) as main component, followed by ß-eudesmol (Dib et al. 2017); this study showed evidence about the signaling mechanism of vasorelaxation induced by AcEO, showing that essential oil acts via L-type calcium channels.

Apium graveolens L. (*A. graveolens*), Apiaceae family. Common name: Celery. This plant has demonstrated antioxidant activity. The n-butylphthalide (NBP) is one of the chemical constituents in celery oil (Houston 2005). Extract of *A. graveolens* was administered as an antihypertensive agent in folk medicine (Gharouni and Sarkati 2000, Moghadam et al. 2013, Vergara-Galicia et al. 2013). Vergara-Galicia et al. (2013) in their study concluded that extracts caused concentration-dependent relaxation in precontracted aortic rings with and without endothelium; for this, the authors suggested that the effect induced by dichloromethane and ethyl acetate extracts from *A. graveolens* is mediated probably by calcium antagonism.

Avena sativa L. *(A. sativa*). Poaceae family. Common names: Oats, avoine, hafer, avena; Avenanthramides are one of the chemical constituents of *A. sativa,* avenanthramides are a group of alkaloids, consisting of an anthranilic acid derivative linked to hydroxycinnamic acid derivative. The three major avenanthramides reported in oat are avenanthramides 1, 3, and 4 (Peterson et al. 2002). These compounds are bioavailable and have anti-inflammatory, anti-atherogenic and antioxidant properties (Peterson et al. 2002, Fu 2015, Martinez-Villaluenga and Penas 2017). Nie et al. (2006) showed that avenanthramides of oats inhibit vascular smooth muscle cell proliferation and enhance nitric the oxide production.

Berberis vulgaris L. (*B. vulgaris*), (berberidaceae family). Common name: barberry. Berberine (BBR), a type of isoquinoline alkaloid, is the major active component of *B. vulgaris,* BBR exhibits several pharmacological activities such as antioxidant activity and has a broad range of therapeutic potential uses including hypertension (Tabeshpour et al. 2017); while Fatehi-Hassanabad et al. (2005), showed that aqueous extract from *Berberis vulgaris* fruit lowers blood pressure in rats (DOCA-induced hypertension) and suggested that the antihypertensive and vasodilatory effects of *B. vulgaris* fruit extract are mainly endothelial-independent.

Morus alba L. (*M. alba*), Moraceae family. Common name: mulberry. Both the fruits and roots have been used traditionally to prevent and treat symptoms associated with cardiovascular disease as hypertension in eastern countries. Rutin and quercetin, were two of the primary components of the leaf (Aminah et al. 2014). In a study Xia et al. (2008) showed that the ethyl acetate extract from leaves (ELM) of *M. alba* on rat thoracic aorta (0.125–32 g/L) induced a concentration-dependent relaxation ($P < 0.01$ *vs.* control), their results also showed that ELM has vasoactive effects and was mediated by inhibition of voltage- and receptor-dependent Ca^{2+} channels; similar to that found by Khan et al. (2014) in anaesthetized rats. On the other hand, Carrizzo et al. (2016) described that *M. alba* extract through its action on eNOS signaling, could act for the regulation of arterial hypertension.

Myrtus communis L. (*M. communis*), Myrtaceae family; common name: Myrtle. Is an evergreen shrub; phytochemical analysis of ethyl acetate extract from this plant revealed the isolation of myricetin-3-O-rhamnoside, a major flavonol in this plant. The results of Bouaziz et al. (2015) study showed that intravenous injection of methanol and ethyl acetate extract at 0.04 to 12 mg/kg body weight, induced a dose-dependent and transitory decrease in SBP, DBP of the anesthetized rats and that the maximum decrease in SBP and DBP was 19 ± 2%; 22 ± 3% and 30 ± 3%; 34 ± 1% for methanol and ethyl acetate extract respectively at the dose of 12 mg/kg.

Passiflora edulis Sims. (*P. edulis*) Passifloraceae family. Common name: passion fruit. Extracts of the family are known to have several important physiological effects in humans, such as anxiolytic, antitussive, antitumoral and antihypertensive properties (Li et al. 2001, Deng et al. 2010, Aguillón et al. 2013, Konta et al. 2014, Restrepo et al. 2014). Passifloraceae genus contains several compounds including alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds (Dhawan et al. 2004, Ichimura et al. 2006, Restrepo et al. 2014); flavonoids as luteolin-6-C-chinovoside and luteolin-6-C-fucoside and aminoacids as gamma aminobutyric acid (GABA) have been isolated from leaves of *P edulis* (Martinez-Villaluenga and Peñas 1991). Flavonoids exhibit diverse biological effects, including inhibition of protein kinase C, inhibition of cyclic nucleotide phosphodiesterase, decrease in Ca^{+2} uptake, and vasodilatory actions (Chan et al. 2000, Ichimura et al. 2006; Tiwaria and Husain 2017). About the antihypertensive properties of *P. edulis* extracts, Ichimura et al. (2006), postulated that the antihypertensive effect of the extract (10 mg/kg) in SHRs might be due mostly to the GABA induced antihypertensive effect and partially to the vasodilatory effect of polyphenols including luteolin.

Zingiber officinale Roscoe. (*Z. officinale*), Zingiberaceae family. Common name: Ginger. It is a well-known spice plant, used traditionally in a wide variety of ailments including management and prevention hypertension (Fugh-Berman 2000, Tabassum and Ahmad 2011, Akinyemi et al. 2013). The main components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and 10-gingerol, these constituents exhibit strong antioxidant activity *in vitro* (Rahmani et al. 2014, Ghayur and Gilani 2005). The crude extract of ginger (Zo.Cr) induced a dose-dependent (0.3–3 mg/kg) fall in the arterial blood pressure of anesthetized rats (Fugh-Berman 2000); this vasodilator effect of Zo.Cr was endothelium-independent. These data and data from different authors indicate that the blood pressure-lowering effect of ginger is mediated through blockade of voltage-dependent calcium channels (Fugh-Berman 2000, Ghayur et al. 2005, Akinyemi et al. 2013). Ginger also inhibited ACE in a dose-dependent manner (25–125 μg/mL) (Akinyemi et al. 2013).

Table 3.1 summarizes and includes these 10 plants and Fig. 3.1 shows some of its active compounds.

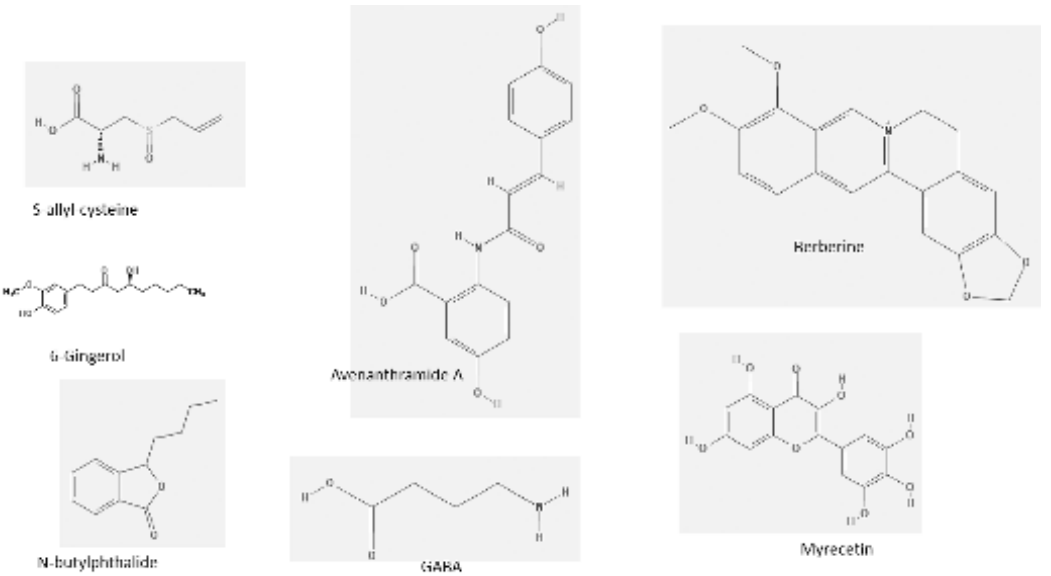


Fig. 3.1 Some active compounds of medicinal plants used in the treatment and management of hypertension. Source: National Center for Biotechnology Information. PubChem Compound Database. Accessed Jan 10, 2018.

Table 3.1 Medicinal plants used for the treatment of hypertension; scientific name, family name, plant parts used.

Scientific name	Family name	Part use	Active compound (AC) or action mechanisms (AM)
Achillea wilhelmsii C Koch.	Asteraceae	Aerial parts	AC = flavonoids and sesquiterpene lactones AM = antioxidant
Allium sativum L.	Amaryllidaceae	Bulb	AC = S-allyl L-cysteine and allicin. AM = endothelial nitric oxide (NO) regulation; ACE inhibition
Annona muricata L.	Annonaceae	Leaves	AC = phenols, alkaloids and acetogenins AM = antagonism of Ca^{2+}
Artemisia campestris L.	Asteraceae	Aerial part	AC = spathulenol, ß-eudesmol AM = L-type Ca^{2+} channels inhibition.
Apium graveolens L.	Apiaceae	Leaves	AC = n-butylphthalide AM = ND
Avena sativa L.	Poaceae/ Gramineae	Whole Cereal	AC = Polyphenols (avenanthramides). AM = free radicals and inflammation reduction, enhances nitric oxide production
Berberis vulgaris L.	Berberidaceae	Fruit, leaves, roots	AC = Berberine and related derivatives AM = Vasoldilatory activity
Morus alba L.	Moraceae	Fruit	AC = Rutin, quercetin. AM = vasorelaxation (voltage- and receptor-dependent Ca^{2+} channels inhibition) Increase NO serum level
Myrtus communis L.	Myrtaceae	Leaves	AC = myricetin-3-Orhamnoside AM = ND
Passiflora edulis Sims.	Passifloraceae	Leaves, fruit	AC = Luteolin-6-C-chinovoside and luteolin-6-C-fucoside; GABA AM = ACE inhibition; antioxidant status enhancement
Zingiber officinale Rosc	Zingiberaceae	Rhizomes	AC = 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol AM = ACE inhibition; blockade of voltage-dependent calcium channels

ND = Not determined.

These plants are used as infusion, decoction, beverages and fresh fruits or raw, but many of them have no scientific validation about their effectiveness or action mechanism or active compound.

Conclusion

There were many plants in folkloric or traditional medicine that were used for the management of hypertension, some had clinical evidence, some had scientific evidence of their mechanism of action and others did not, but it is undeniable that these plants are the best source of drugs and therefore need to be studied in depth.

This work provides knowledge in medicinal plants used for hypertension treatment; describes some metabolites from these plants to inhibit ACE and calcium channel, or to increased nitric oxide release or/ and vasorelaxation.

References

Aguillón, J., Maldonado, M.E, Loango, N., Arango, S.S. and Landázuri, P. 2013. Antioxidant and antiproliferative activity of ethanolic and aqueous extracts from leaves and fruits juice. *Passiflora edulis*. Perspect Nutr. Humana 15: 13–25.

Ahmad, L., Semotiuk, A., Zafar, M., Ahmad, M., Sultana, S., Liu, Q-R. and Yaseen, G. 2015. Ethnopharmacological documentation of medicinal plants used for hypertension among the local communities of DIR Lower, Pakistan. J. Ethnopharmacol. 175: 138–146.

Ahmed, N., Martinelli, A. and Kiruthi, C. 2015. Chapter 20 – Antihypertensive drugs. In book: Side Effects of Drugs Annual 37: 227–236.

Alsabbagh, M.H.D.W., Lemstra, M., Eurich, D., Lix, L.M., Wilson, T.W., Watson, E. and Blackburn, D.F. 2014. Socioeconomic status and non-adherence to antihypertensive drugs: a systematic review and meta-analysis. Value in Health 17(2): 288–296.

Akinyemi, A.J., Ademiluyi, A.O. and Oboh, G. 2013. Aqueous extracts of two varieties of ginger (*zingiber officinale*) inhibit angiotensin I–converting enzyme, iron (II), and sodium nitroprusside-induced lipid peroxidation in the rat heart *in vitro*. J. Med. Food 16(7): 641–646.

Aminah, S., Suwaldi, S., Fudholi, A. and Wahyono, W. 2014. The effect of encapsulated mulberry (*Morus alba* L.) leaves extract on arterial blood pressure in rats. Trad. Med. J. 19(3): 149–155.

Asgary, S., Naderi, G.H., Sarrafzadegan, N., Mohammadifard, N., Mostafavi, S. and Vakili, R. 2000. Antihypertensive and antihyperlipidemic effects of *Achillea wilhelmsii*. Drugs Exp. Clin. Res. 26(3): 89–93.

Ashraf, R., Khan, R.A., Ashraf, I. and Qureshi, A.A. 2013. Effects of *Allium sativum* (garlic) on systolic and diastolic blood pressure in patients with essential hypertension. Pak. J. Pharm. Sci. 26(5): 859–63.

Baharvand-Ahmadi, B., Bahmani, M., Tajeddini, P., Rafieian-Kopaei, M. and Naghdi, N. 2016. An ethnobotanical study of medicinal plants administered for the treatment of hypertension. J. Renal. Inj. Prev. 5(3): 123–128.

Balakumar, P. and Jagadeesh, G. 2014. A century old renin-angiotensin system still grows with endless possibilities: AT1 receptor signaling cascades in cardiovascular physiopathology. Cell Signal 26(10): 2147–2160.

Bouaziz, A., Khennouf, S., Abu zarga, M., Abdalla, S., Baghiani, A. and Charef, N. 2015. Phytochemical analysis, hypotensive effect and antioxidant properties of *Myrtus communis* L. growing in Algeria. Asian Pac. J. Trop. Biomed. 5(1): 19–28.

Carrizzo, A., Ambrosio, M., Damato, A., Madonna, M., Storto, M., Capocci, L. et al. 2016 *Morus alba* extract modulates blood pressure homeostasis through eNOS signaling. Mol. Nutr. Food Res. 60(10): 2304–2311.

Chan, E.C., Pannangpetch, P. and Woodman, O.L. 2000. Relaxation to flavones and flavonols in rat isolated thoracic aorta: mechanism of action and structure activity relationships. J. Cardiovasc. Pharmacol. 35: 326–333.

Charlton, M. and Thompson, J.P. 2015. Drugs acting on the heart: Antihypertensive drugs. Anaesth. Intensive Care Med. 16(5): 227–231.

Chockalingam, A., Campbell, N.R. and George, F.J. 2006. Worldwide epidemic of hypertension. Can. J. Cardiol. 22(7): 553–555.

Deng, J., Zhou, Y., Bai, M., Li. H. and Li, L. 2010. Anxiolytic and sedative activities of *Passiflora edulis* f. flavicarpa. J. Ethnopharmacol. 128(1): 148–153.

Dhawan, K., Dhawan, S. and Sharma, A. 2004. *Passiflora*: a review update. J. Ethnopharmacol. 94: 1–23.

Dib, I., Fauconnier, M.L., Sindic, M., Belmekki, F., Assaidi, A., Berrabah, M. et al. 2017. Chemical composition, vasorelaxant, antioxidant and antiplatelet effects of essential oil of *Artemisia campestris* L. from Oriental Morocco. BMC Complem. Alternat. Med. 17(82): 2–15.

Dutra, R.C., Campos, M.M., Santos, A.R.S. and Calixto. J.B. 2016. Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. Pharmacol. Res. 112: 4–29.

Eddouks, M., Ajebli, M. and Hebi, M. 2017. Ethnopharmacological survey of medicinal plants used in Daraa-Tafilalet region (Province of Errachidia). Morocco. J. Ethnopharmacol. 198: 516–530.

Falzon, C.C. and Balabanova, A. 2017. Phytotherapy: An Introduction to Herbal Medicine. Prim. Care 44(2): 217–227.

Fatehi-Hassanabad, Z., Jafarzadeh, M., Tarhini, A. and Fatehi, M. 2005. The antihypertensive and vasodilator effects of aqueous extract from *Berberis vulgaris* fruit on hypertensive rats. Phytother. Res. 19(3): 222–225.

Ferreira, L.G., Evora, P.R.B., Capellini, V.K., Albuquerque, A.A., Carvalho, M.T.M., Da Silva Gomes, R.A., Parolini, M.T. and Celotto, A.C. 2018. Effect of rosmarinic acid on the arterial blood pressure in normotensive and hypertensive rats: role of ACE. Phytomedicine 38: 158–65.

Fu, J., Zhu, Y., Yerke, A., Wise, M.L., Johnson, J., Chu, Y. and Sang, S. 2015. Oat avenanthramides induce heme oxygenase-1 expression via Nrf2-mediated signaling in HK-2 cells. Mol. Nutr. Food Res. 59: 2471–2479.

Fugh-Berman, A. 2000. Herbs and dietary supplements in the prevention and treatment of cardiovascular disease. Prev. Cardiol. 3(1): 24–32.

Gharouni, M. and Sarkati, A. 2000. Application of *Apium graveolens* in treatment of hypertension. J. Tehran. Univ. Med. Sci. 3: 67–69.

Ghayur, M.N. and Gilani, A.H. 2005. Ginger lowers blood pressure through blockade of voltage-dependent calcium channels. J. Cardiovasc. Pharmacol. 45(1): 74–80.

Houston, M.C. 2005 Nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. Prog. Cardiovasc. Dis. 47(6): 396–449.

Gerwick, W.H. 2017. Plant sources of drugs and chemicals. In book: Reference module in life sciences. DOI:10.1016/B978-0-12-809633-8.02306-2.

Ichimura, T., Yamanaka. A., Ichiba, T., Toyokawa, T., Kamada, Y., Tamamura, T. and Maruyama, S. 2006 Antihypertensive Effect of an extract of *Passiflora edulis* rind in spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. 70(3): 718–721.

James, P.A., Oparil, S., Carter, B.L., Cushman, W.C., Dennison-Himmelfarb, C., Handler, J. and Ortiz, E. 2014. Evidence-based guideline for the management of high blood pressure in adults. JAMA, 311(5): 507–520.

Khan, M., Khan, A-U., Rehman, N-Ur. and Gilani, A-H. 2014. Blood pressure lowering effect of *Morus alba* is mediated through Ca^{++} antagonist pathway. Int. J. Pharmacol. 10(4): 225–230.

Kiriyama, A., Honbo, A., Nishimura, A., Shibata, N. and Iga, K. 2016. Pharmacokinetic-pharmacodynamic analyses of antihypertensive drugs, nifedipine and propranolol, in spontaneously hypertensive rats to investigate characteristics of effect and side effects. Regul. Toxicol. Pharm. 76: 21–29.

Konta, E.M., Almeida, M.R., do Amaral, C.L., Darin, J.D., de Rosso. V.V., Mercadante, A.Z., Antunes, L.M. and Bianchi, M.L. 2014. Evaluation of the antihypertensive properties of yellow passion fruit pulp (*Passiflora edulis* Sims f. flavicarpa Deg.) in spontaneously hypertensive rats. Phytother. Res. 28(1): 28–32.

Landazuri, P., Loango, N. and Restrepo, B. 2017. Medicinal plants used in the management hypertension. J. Anal. Pharm. Res. Med. 5(2): 5–7.

Lee, S.Y. and Hur, S. 2017. Antihypertensive peptides from animal products, marine organisms, and plants. Food Chem. 228: 506–517.

Leung, A.A., Daskalopoulou, S.S., Dasgupta, K., McBrien, K., Butalia. S., Zarnke, K.B. and Rabi, D.M. 2017. Hypertension Canada's 2017 Guidelines for diagnosis, risk assessment, prevention, and treatment of hypertension in adults. Can. J. Cardiol. 33(5): 557–576.

Li, H., Zhou, P., Yang, Q., Shen, Y., Deng, J., Li, L. and Zhao, D. 2001. Comparative studies on anxiolytic activities and flavonoid compositions of *Passiflora edulis* 'edulis' and *Passiflora edulis* 'flavicarpa'. J. Ethnopharmacol. 16: 133(3): 1085–1090.

Lima Prando, T.B., Barboza, L.N., Gasparotto, F.M., De Oliveira Araujo, V., Signor Tirloni, C.A., De Souza, L.M. and Gasparotto Junior, A. 2015. Ethnopharmacological investigation of the diuretic and hemodynamic properties of native species of the Brazilian biodiversity. J. Ethnopharmacol. 174: 369–378.

Lin, T., Yen, H., Chiang, J., Sun, M. and Chang, H. 2017. The use of Chinese herbal medicine as an adjuvant therapy to reduce incidence of chronic hepatitis in colon cancer patients: A Taiwanese population-based cohort. J. Ethnopharmacol. 202: 225–233.

Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A, Buhm. M. and Wood, D.A. 2013. ESH/ESC guidelines for the management of arterial hypertension: The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur. Heart J. 34(28): 2159–2219.

Mareck, U., Herrmann, K., Galensa. R. and Wray, V. 1991. The 6-C-chinovoside and 6-C-fucoside of luteolin from *Passiflora edulis*. Phytochemistry 30: 3486–3487.

Martınez-Villaluenga, C. and Peñas. E. 2017. Health benefits of oat: current evidence and molecular mechanisms. Current Opinion. Food Sci. 14: 26–31.

Medina-Remón, A., Tresserra-Rimbau, A., Pons, A., Tur, J.A., Martorell, M., Ros, E. and Lamuela-Raventos, R.M. 2015. Effects of total dietary polyphenols on plasma nitric oxide and blood pressure in a high cardiovascular risk cohort. The PREDIMED randomized trial. Nutr. Metab. Cardiovasc. Dis. 25(1): 60–67.

Moghadam, M.H., Imenshahidi, M. and Mohajeri, S.A. 2013. Antihypertensive effect of celery seed on rat blood pressure in chronic administration. J. Med. Food 16(6): 558–563.

Nangia, R., Singh, H. and Kaur, K. 2016. Prevalence of cardiovascular disease (CVD) risk factors. Med. J. Armed. Forces India 72(4): 315–319.

Niazmand, S., Harandizadeh, F., Mahmoudabady, M., Hasanzadeh. M. and Fereidouni, E. 2014. Mechanism of vasorelaxation induced by *Achillea wilhelmsii* in rat isolated thoracic aorta. Adv. Biomed. Res. 3(91): 1–7.

Nie, L., Wise, M.L., Peterson, D.M. and Meydani, M. 2006. Avenanthramide, a polyphenol from oats, inhibits vascular smooth muscle cell proliferation and enhances nitric oxide production. Atherosclerosis 186(2): 260–266.

Nishioka, R., Kinoshita, S., Shiibashi, M., Shimazu, T., Nakazato, Y., Yamamoto, T. and Araki, N. 2015. Evaluation of the differences in the effects of antihypertensive drugs on blood pressure variability by 24-hour ambulatory blood pressure monitoring in chronic Ccerebrovascular disease. J. Stroke Cerebrovasc. Dis. 24(8): 1848–1854.

Nunes, M.G.S., Bernardino, A. and Martins, R.D. 2015. Use of medicinal plants by people with hypertension. Rev. RENE 16(6): 775–781.

Nwokocha, C.R., Owu, D.U., Gordon, A., Thaxter, K., McCalla, G., Ozolua, R.I. and Young. L. 2012. Possible mechanisms of action of the hypotensive effect of *Annona muricata* (soursop) in normotensive Sprague–Dawley rats. Pharm. Biol. 50(11): 1436–41.

Olowokudejo, J.D., Kadiri, A.B. and Travih, V.A. 2008 An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. Ethnobotanical. Leaflets 12: 851865.

Patel, S. and Patel, J.K. 2016. A review on a miracle fruits of *Annona muricata*. J. Pharmacogn. Phytochem. 5(1): 137–148.

Patel, S., Rauf, A., Khan, H. and Abu-Izneid, T. 2017. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. Biomed. Pharmacother. 94: 317–325.

Peterson, D.M., Hahn, M.J. and Ammonds, C.L. 2002. Oat avenanthramides exhibit antioxidant activities *in vitro*. Food Chem. 79(4): 473–478.

Petroni, L.M., Huffman, M.A. and Rodrigues, E. 2017. Medicinal plants in the diet of woolly spider monkeys (*Brachyteles arachnoides*, E. Geoffroy, 1806)-a biorational for the search of new medicines for human use?. Rev. Bras. Farmacogn. 27(2): 135–142.

Polat, R., Cakilcioglu, U., Kaltalioglu. K., Ulusan, M.D. and Turkmen, Z. 2015. An ethnobotanical study on medicinal plants in Espiye and its surrounding (Giresun-Turkey). J. Ethnopharmacol. 163: 1–11.

Popovi, Z., Mati, R., Bojovi, S., Stefanovi, M. and Vidakovi, V. 2016. Ethnobotany and herbal medicine in modern complementary and alternative medicine: An overview of publications in the field of I & C medicine 2001–2013. J. Ethnopharmacol. 181: 182–192.

Rahmani, A.H., Al shabrmi, F.M. and Aly, S.M. 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int. J. Physiol. Pathophysiol. Pharmacol. 6(2): 125–136.

Rawat, P., Singh, P.K. and Kumar, V. 2016. Anti-hypertensive medicinal plants and their mode of action. J. Herbal. Medicine 6(3): 107–118.

Restrepo, R.A., Loango, N., Moncada, M.V. and Landázuri, P. 2013. Angiotensin-converting enzyme inhibitory activity of *Passiflora edulis* flavicarpa and *Petroselinum crispum* (Mill) Fuss. Br. J. Pharm. Res. 3(4): 776–785.

Restrepo, R.A, Nieto, O.A., Aristizabal, J. and Landázuri, P. 2014. Angiotensin-converting enzyme inhibition by *Phthirusa pyrifolia* (Kunth) Eicher. World J. Pharm. Sci. 3(6): 352–363.

Ried, K. and Fakler, P. 2014. Potential of garlic (*Allium sativum*) in lowering high blood pressure: mechanisms of action and clinical relevance. Integr. Blood Press. Control. 7: 71–82.

Shayganni, E., Bahmani, M., Asgary, S. and Rafieian-Kopaei, M. 2016. Inflammaging and cardiovascular disease: Management by medicinal plants. Phytomedicine 23(11): 1119–1126.

Tabassum, N. and Ahmad, F. 2011. Role of natural herbs in the treatment of hypertension. Phcog. Rev. 5(9): 30–40.

Tabeshpour, J., Imenshahidi, M. and Hosseinzadeh, H.A. 2017. Review of the effects of *Berberis vulgaris* and its major component, berberine, in metabolic syndrome, Iran. J. Basic. Med. Sci. 20(5): 557–568.

Textor, S.C. 2017. Renal arterial disease and hypertension. Med. Clin. North Am. 101(1): 65–79.

Tiwaria, S.C. and Husain, N. 2017. Biological activities and role of flavonoids in human health—a review. Indian J. Sci. Res. 12(2): 193–196.

Torlasco, C., Faini, A., Makil, E., Ferri, C., Borghi, C., Veglio, F. and Parati, G. 2017. Cardiovascular risk and hypertension control in Italy. Data from the 2015 World Hypertension Day. Int. J. Cardiol. 243: 529–532.

Vardanyan, R., Hruby, V., Vardanyan, R. and Hruby, V. 2016. Chapter 22 Antihypertensive drugs. pp. 329–356. *In*: Synthesis of Best-Seller Drugs.

Vergara-Galicia, J., Jimenez-Ramirez, L.Á., Tun-Suarez, A., Aguirre-Crespo, F., Salazar-Gómez, A., Estrada-Soto, S. et al. 2013. Vasorelaxant activity of extracts obtained from *Apium graveolens*: Possible source for vasorelaxant molecules isolation with potential antihypertensive effect. Asian Pac. J. Trop. Biomed. 3(10): 776–779.

WHO 2013. A global brief on hypertension. A global brief on hypertension 40.

World Heart Federation. 2015. Cardiovascular disease risk factors - Hypertension World Heart Federation 1–2. Retrieved from http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/hypertension/

Xia, M., Qian, L., Zhou, X., Gao, Q., Bruce, I.C. and Xia, Q. 2008. Endothelium-independent relaxation and contraction of rat aorta induced by ethyl acetate extract from leaves of *Morus alba* (L.). J. Ethnopharmacol. 120(3): 442–446.

Yan, J.K., Ding, L.Q., Shi, X.L., Donkor, P.O., Chen, L.X. and Qiu, F. 2017. Megastigmane glycosides from leaves of *Eucommia ulmoides* Oliver with ACE inhibitory activity. Fitoterapia 116: 121–125.

4

Plants Used for Central Nervous System Disorders by Brazilian Indians

Priscila Yazbek,[1,*] *Thamara Sauini*,[1] *Fernando Cassas*,[1] *Giuseppina Negri*[2] and *Eliana Rodrigues*[1]

Introduction

Ethnopharmacology is a recent discipline in the Academia. Although the term ethnopharmacology had firstly appeared in 1967, "*Ethnopharmacological search for new psychoactive drugs*", the 'idea' of this discipline had already been presented in 1924 by Louis Lewin in his work entitled 'Phantastica'.

Originally, ethnopharmacology was defined as a science that sought to understand, from field works, the natural resources' (plants, animals) universe used as drugs from human groups' point of view. One of these definitions was given by Schultes (1988) "*...ethnopharmacology, sub area of ethnobotany, is a recent discipline in the academic world, and it refers to medical or pseudomedical use of plants and animals by pre-literate societies*".

However, over time, some researchers have defined it in the context of pharmacological and phytochemical studies and not field work. That is, the plants or animals indicated during the field work among Indians, for example, should be investigated by these sciences in order to prove or not their 'empirical' use; however, the field work itself would not be considered an ethnopharmacological study. One of these definition was employed by Holmstedt and Bruhn as "*...the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by men"* (Holmstedt and Bruhn 1983). This view has been reinforced by current concepts, such as the International Society for Ethnopharmacology (2005), *"... interdisciplinary study of the physiological actions of plants, animals and other substances used in indigenous medicines of past and present cultures"*.

These latter definitions, somehow, establish a link between 'traditional and/or popular' and 'academic' knowledge. In this way, data from field work studies must be tested and proven or not by academic science. However, this was not the original idea which intended to describe other cultures' medicine. The uses of plants and animals, as well as their effects, were seen in the context of a particular human group, whether or not official medicine could use it for its benefit. The historical trajectory of ethnopharmacology definitions makes us think that ethnopharmacology is only science if tested and approved by our science; not seeing or recognizing other cultures knowledge as a science in itself.

[1] Center for Ethnobotanical and Ethnopharmacological Studies-Institute of Environmental Sciences, Chemical and Pharmaceutical, Federal University of São Paulo, SP, Brazil.
[2] Federal University of São Paulo, SP, Brazil.
* Corresponding author: pbyazbek@gmail.com

Ethnopharmacology does not deal with superstitions, but with popular knowledge related to traditional systems of medicine. To appreciate this knowledge it must be admitted, without prejudice, as a body of knowledge, a product of the human intellect (Elisabetsky 2003). Therefore, traditional and/or popular knowledge does not need to be validated by 'our' science to be legitimate. It exists regardless of its judgment, criticism or evaluation by 'our medicine'.

Considering that ethnopharmacology, inserted in the context of ethnobiology, is the study of developed knowledge and conceptualizations by any culture about its medical practices, the relations between human populations and medicinal plants and the remedies used in traditional medical systems (Elisabetsky 2003, Albuquerque 2005). This chapter aims to highlight the importance of studying indigenous communities, their knowledge and culture in order to guide future pharmacological studies of plants with possible action on the Central Nervous System (CNS).

Ethnopharmacology: approaches and cultural context

Ethnopharmacology includes diverse approaches as ethnopharmacological surveys carried out during field work among diverse cultures in order to rescue aspects of local medicine; studies that relate human displacement to popular and/or traditional knowledge; studies that depart from popular and/or traditional knowledge to rescue plants (and other resources) that present use restriction, bringing elements to evaluate risks in these medicines consumption; studies comparing knowledge among different cultures (even in the same biome); and finally studies that depart from ancient literature reports to rescue the knowledge of medicinal resources used centuries ago.

The development of these approaches is based on methods and techniques of anthropology (ethnography) and biology (botany and zoology). Ethnography is used to record the complexity of local medicines, as mentioned above, including medicinal uses of plants and animals in most cases (but also fungi, minerals, algae and other substances). While botanical and zoological methods are used to collect the resources (plants and animals) mentioned through the indications (Rodrigues and Otsuka 2012). As in ethnobotanical studies, methods and analyses of data may be qualitative or quantitative, or—more rarely—it can involve both approaches. For more details, see (Bernard 1988, Alexiades 1996).

According to Balick and Cox (1996) two different approaches can be followed in order to select a plant species for investigation. The first one is the random selection where any plant can be choosen for the screening of its substances without taking into account its taxonomic kinship, intrisic qualities or ethnobotanical context. Even though some drugs, such as taxol used to treat ovarian and breast cancer, have been discovered through random selection, this approach has low success rates. The other is the target approach which can be done by various ways as the selection of a plant from a taxonomic family or genus with known medicinal properties, a particular habitat or assertive characteristics as immunity to predation by animals, and a plant indicated through an ethnobotanical survey. When both approaches are compared, authors contest that drug discovery are more likely to succeed when ethnobotanical surveys are conducted.

Svetaz et al. (2010) tested plant species indicated by Latin America traditional medicine against fungal pathogens and concluded that plants indicated by the communities inhibited growth of pathogenic fungi in a higher percentage than the plants choosen randomly (40 against 21%). The study conducted by Gyllenhaal et al. (2012) compared the bioassay activity of plants selected through the random approach and the ones indicated by ethnomedicine from Laos and Vietnam and they observed a higher activity rate in the plants collected according to the indications. Although some of the specific uses were not confirmed by the assays.

The lack of expected effects on pharmacological tests may occur because laboratory tests do not address cultural contexts in which medicinal species are used, in other words, they are not accompanied by local practices and beliefs that provide the meaning to medicinal plants, animals, and minerals. Therefore, even a species having active therapeutic principles as indicated by a certain culture, do not act alone on healing contexts. The cultural therapeutic meaning contributes to its effect. Thus, pharmacological tests should be conducted considering this cultural context, avoiding misinterpretations of inefficiency for having been tested separately from beliefs and practices involved in their uses (Moerman 2007, Reyes-Garcia 2010).

Besides which, a plant's efficacy failure on clinical tests can be related to translation gaps from "emic[1]" to "etic"[2] terms. Often ethnopharmacological indications refer to "local diseases" or cultural-bound syndromes that require rigorous observations and interpretation so they can be correlated with the correct "etic" term used by conventional medicine. Nevertheless, the correct interpretation can be very hard to be achieved, since cultural-bound systems often link clinical manifestations to conditions influenced by cultural factors (Tseng 2006, Quinlan 2010), that do not always take into account conventional medicine.

Thus as defended by Pagani et al. (2017): *"The correlation between the "emic" terms used by the local populations, with their correspondent "etic" terms and the natural resources used for the healing, may give insights to guide further pharmacological studies. One main role of ethnopharmacology is to address these correlations, since they are the bases for suggesting the potential bioactivity of these resources*".

The evolution of ethnopharmacology as a discipline has taken great strides in the understanding of traditional cosmologies and it may contribute to guide the development of new drugs. Some papers have brought discussions on the relevance of theoretical and methodological contributions, as well as the importance of interdisciplinarity to the success of studies conducted (Waldstein 2006, de Oliveira et al. 2009, Reyes-Garcia 2010).

Reyes-Garcia (2010), as an example, draws attention for the potential contribution of social sciences and traditional knowledge systems, defined by: "*[...] the knowledge of resource and ecosystem dynamics and associated management practices existing among people of communities that, on a daily basis and over long periods of time, interact for their benefit and livelihood with ecosystems. The term does not merely refer to information about human uses of plants and animals. Rather, it includes a system of classifications, a set of empirical observations about the local environment, and a system of resource use and management. It also includes believes in non-human beings (i.e., spirits, ancestors, ghosts, gods) and on how they relate to society*".

Moreover, many papers have been published about plants' knowledge transmission, exchange of knowledge between different communities and between traditional and conventional medicines, meaning of cultural-bound syndromes and hypothesis to plants' selection (Albuquerque and Lucena 2005, Leonti 2011, de Medeiros et al. 2015, Pagani et al. 2017). These questions are essential to understand the use of certain species in function of others, forms of use, dosage, choice of plants and, in later stages, these informations can contribute for the reproduction of clinical assays that resemble cultural practices, since the efficacy of medicinal plants are not only due to the plants, but also due to the cultural contexts they are inserted.

In this case, in addition to register and value cultural diversity and traditional knowledge, the need of this knowledge guides scientific and technological development of drugs through the study of bioactive potential, allowing flora to provide drugs widely used in the clinic, for example, artemisinin, colchicine, emetin, forskolin, rutin, taxol and vincristine (Cechihinel Filho and Rosendo 1998). However, in most cases, methods of pharmacology are lacking in achieving the logic of these medicines, which draws attention to the cultural context understanding in order to consider these cultural elements for conduction of clinical tests.

Indigenous people and shamanism

Brazil began its history in the year 1503 with the discovery of a new world by the Portuguese. However, this new world had already inhabitants who, according to estimates, totaled approximately 8 million people scattered in at least 126 ethnic groups. The Tupi-Guaranis, who lived in the Atlantic forest bordering the ocean, had already called it the territory of Pindorama, for example. Currently, according to the Brazilian government, there are about 896,900 indigenous people distributed in 305 ethnic groups spread across Brazil's six main biomes (FUNAI 2017). There are 505 indigenous territories recognized by the National Indian Foundation (FUNAI 2017), corresponding to 106.7 million hectares, or 12.5% of the country's territory. The largest concentration of these people is in the north, totaling 251.9 thousand indigenous (48.7%), where the largest population is the Yanomami (25.7 thousand) in the states of Amazonas and Roraima, living in the Amazon forest biome (FUNAI 2017).

[1] Attempt to describe the behavioral system of a given culture using its own terms.
[2] Attempt to describe the behavioral system of a given culture using academic terms.

These indigenous people, or nations, have an ancestral origin which, according to the two main theories of peoples' migration to Americas, have settled in the region between 12 and 14 thousand years ago and continue to survive to this day. Throughout the development of each culture we can consider general topics in common for its establishment and survival, as: coexistence in family nuclei; religious practices; healing practices; music; dance and manufactured artifacts (technology). All these topics are practised by the 305 ethnic groups that differ in most ways, primarily, by the language and the biome where they are located.

The country is world renowned for its biodiversity, and it is among the 17 megadiverse in the world, which together account for 70% of the planet's biodiversity (Scarano 2009). The six main Brazilian biomes are: Atlantic forest, Amazonian forest, Cerrado, Caatinga, the Pampa and the Pantanais-Matogrossenses macro biome (IBGE 2017). It has the richest flora in the world, with approximately 55,000 species of superior plants already described (Giulietti et al. 2005).

In each of these biomes there are endemic and native plant species. Their estimated numbers makes it possible to measure the size of its biodiversity. For example, the Atlantic Forest has around 20 thousand species of which 8 thousand are endemic, Cerrado possesses 11 thousand species of which 6 thousand are endemic. While according to the study published by Giulietti et al. (2005), the Amazon possesses 30 thousand species, Caatinga 5 thousand species, and Pantanais-Matogrossenses 2 thousand species. In this way we can understand that relation bioma versus human group, considering the 305 ethnicities and biomes where they are established over the millennia, results in a great variety of cultural knowledge and understanding about fauna and flora. Mainly access to these communities are very hard, with no roads connecting them to cities, and thus makes the access of public and/or private health very tough, contributing to the strengthening of traditional and popular local medicines (Rodrigues and Otsuka 2012).

However, in the last decades, the accelerated globalization process, global economy development and the modernization of rural areas have been promoting the homogenization of societies, and, consequently, cultural changes of traditional practices and knowledge. The consequences of these processes remain under-explored (Cavalli-Sforza and Feldman 1981, Balick and Cox 1996).

Urbanization can impact local knowledge of these communities in two different ways, as discussed by de Medeiros et al. (2015). The first one suggests that after having contact with external resources the people give up their healing practices and allopathic medicines are replacing medicinal plants, since the element incorporated is considered competitive and not complementary to a population knowledge. In this case, and as time passes, the knowledge practices present before the urbanization process may be replaced. The other possibility is the coexistence of both systems. In this case, new elements are added in the medical system and do not replace medicinal plants (Soldati and Albuquerque 2012). People will still use the traditional practices and medicinal plants, but they will also administer allopathic medicine or drugs prescribed by the doctors.

Both situations illustrate that knowledge is always changing, incorporating elements from other cultures and sometimes replacing practices and customs. In this context, we highlight the importance of "learning" from these communities techniques for the selection of medicinal plants and not only listing the medicinal plants used by them.

In the case of Brazil, 305 indigenous ethnicities identified, as it is evident, until their first contact with the Advanced Industrial Society Ideology (Marcuse 1973, FUNAI 2017) or with the culture of non-Indians, established different relations in their everyday life. All intellectual and natural material used to come from tradition or ancestry and natural resources were native of forests in which they lived. Since clothing, food, tools, medicines and utensils used to come only from their culture and region.

In this way, we can note the intrinsic relationship, mystical and the close proximity with the forest and the relations that were established in the values of each community subject. Generally, we can establish the main elements that form the social organization of these peoples making analogies with the non-Indian culture. In the villages (neighborhoods, towns, communities, cities) there are Pajés (political leaders, groups' representatives), hunters (meat producers, farmers), planters and collectors (farmers, rural producers), warriors (police, military, security), singers and rattle players (musicians, instrumentalists and artists), family members who teach the youngest (teachers, educators) and Shamans (doctors, pharmacists, nurses, health professionals).

Shamans and Shamanism are the names designated by non-Indians to healing practitioners of indigenous communities. Since each culture establishes a different language, the names are also different.

The practice differs from non-Indian culture because beyond therapeutic practice and cure of diseases, it involves religious aspects that are instrinsically linked to the process. It is important to highlight at this point that, all the daily practices of Indians are linked to a religious and spiritual symbolism almost entirely. Thus, decisions made on a daily basis are not interpreted according to the logic of empirical-theoretical-technical rationality of western thought, but rather to a non-linear abstraction of facts interpretation, resulting in a logic contained in metaphysical or mystical universes. To understand this type of thinking and how relationships are established we will quote the example below:

The Krahô indigenous nation belongs to the *macro-jê* trunk, *jê* family and *timbira* language. In their ascension, the Krahô suffered influences of the ethnic groups Canela, Xerente and Apinayé. Currently, there are approximately 3,000 Krahôs, distributed in 19 villages and occupying 302,533 hectares in the cerrado of Goiatins and Itacajá municipalities, at the north of Tocantins state. The villages are located far from the nearest towns. In addition to not having public transport, the roads leading to them are precarious and difficult to access, especially during the winter season, when there are strong rains (between October and April). These factors reinforce the need to use plants for diseases' treatments. According to Melatti (1978), the Krahô are divided in two groups, one of them is the pair-*wakmẽye* (summer) and the other the pair-*katamye* (winter). These divisions are characterized by a series of ritual and symbolic acts that distinguish members of the half of each pair:

- *Wakmẽye* whose symbols are: day; dry season; summer; east of the village courtyard; straws for light-colored garnishment; vertical stripes of body painting and parakeet;
- *Katamye* has the following symbols: night; rainy season; west; outskirts of the village; straws for dark-colored garnishment; horizontal stripes and sucuruju snakes.

According to Rodrigues (2001), in the same way, the Krahô classify the plants and animals as belonging to one or the other seasonal half, depending on the symbology that each one represents. Thus, plants that flourish or fruit in the summer, belong to the *wakmẽye* half and those that bloom or fruit in the winter, belong to the *katamye*. They explain that mid-summer plants are "more powerful" remedies than those of winter, as rainwater, which is frequent in winter, they say, "dilutes" the "force" of these plants. According to the interviewees, "*wajaca* is the person recognized by the Krahô people as holder of herbal remedies and healing processes knowledge, by which he receives instructions and assistance from his respective *pahí*, spiritual guide, generally represented by the spirit of an animal, plant, mineral, object or even of someone that has passed away. He can cure or kill other person, acting as a *wajaca* or as a sorcerer. Smoking act can help to communicate with their *pahí* or to produce more power at the time of healing".

The most striking characteristics of this ethnic group are the richness of rituals and the use of medicinal plants in healing processes (Melatti 1978). The anthropologist laments the lack of technical investment in the collection and taxonomic identification of vegetal species used in magical rituals by these Indians on that occasion, since:

> *"I found it was useful to identify those plants that the Krahô use for magical purposes, since a laboratory analysis might prove that some of them actually contain some substance that would produce the effects sought by the indians"*
>
> (translated from Melatti 1978).

In his work "Ritos de uma tribo Timbira" (Rites of a Timbira tribe), the author describes more than 40 rites observed among this group, most of them involving the use of animals and plants. In this sense, 20 years after this work, two researchers dedicated themselves to the survey of medicinal plants used during rituals by this ethnic group. They described about 138 plants divided into 10 classes related to Central Nervous System disorders (Rodrigues and Carlini 2005, 2006).

As mentioned above, the use of plants with possible effects on the central nervous system by Indians has a strict relation with shamanism, since, as defended by Schultes and Hofmann (1993), indigenous communities do not differentiate between physiological and supernatural causes of diseases. Many ethnicities believe that illnesses are the result of some interference, pendency, or trouble in the spirit world. Therefore, they believe that effective cure of diseases can only be carried out in this plan and for this they must access it with the aid of plants, in most cases, psychoactive plants (Júnior et al. 2015).

Also on the spiritual level, the *wajacas* of Krahô ethnicity access their respective *pahí* and shamans from other ethnic groups contacting their spiritual guides through dreams, rituals and the use of plants, usually hallucinogenic, that act on the CNS. In these moments, spiritual revelations indicate the use of effective plants for different diseases and for each sick person (Rodrigues 2001, Júnior et al. 2015), leading to a treatment that considers specificities and characteristics of each patient, that is, an individualized treatment. In the case of the Krahô Indians, each *wajaca* uses different plants for the same condition, this differentiation is due to the different guides accessed by each curator who teach different applications for plants species, as observed by Rodrigues and Carlini (2006). However, each indigenous ethnicity has a different relationship with the spiritual plan and the spiritual guides, and few ethnopharmacological surveys detail the contexts behind the curators' choice of medicinal plants.

Because access to the spiritual plan is of great importance for diseases' treatments in indigenous communities, and considering that several medicinal plants are indicated for the same pathological conditions, it is expected that the number of plants indicated for the CNS by these cultures will be ample. Whereas Brazil has many endemic species in its biomes, the use of these plants by these communities can provide a great variety of potential bioactive compounds for the CNS.

However, if the intention is to investigate whether these plants are effective in diseases treatments designated by biomedicine, we must understand the different ways of seeing, recognizing and approaching diseases by these people, considering the use contexts of these species and their cultural meanings.

Plants used in Central Nervous System disorders by Brazilian Indians

In 2006, Rodrigues et al. carried out a bibliographical review in order to analyze species of plants used by several Brazilian indigenous ethnicities, as well as the relation between their chemical constituents and their uses for Central Nervous System (CNS) disorders. These data were updated, and among studies found from the 70s to the present day, 358 plant species, belonging to 97 botanical families, used by Indians for different diseases were listed. These indications are available in 36 publications (for more information see Rodrigues et al. 2006, Cunha et al. 2012, Kffuri et al. 2016).

Of the 97 taxonomic families listed, the most cited were: Fabaceae (60 registered species), Asteraceae (23), Rubiaceae (18), Poaceae (14), Euphorbiaceae (14), Bignoniaceae (11), Apocynaceae (10), Solanaceae (10), Lamiaceae (10), Piperaceae (10), Rutaceae (10), Verbenaceae (10), Cyperaceae (7) and Moraceae (7). The families Fabaceae, Euphorbiaceae, Asteraceae, Bignoniaceae and Rubiaceae were also the most cited during the research conducted by Rodrigues and Carlini (2005), who have also investigated the plants used for CNS disorders.

According to Socio-Environmental Institute (ISA 2017), 48 Brazilian indigenous ethnic groups have part of their population residing in other countries. In this manner, studies described here were carried out with a total of 27 indiginous ethnic groups, who inhabit four of the Brazilian biomes (Amazon Forest, Caatinga, Cerrado and Atlantic Forest), and some also inhabit other countries of South America; such as the Yanomami, whose geographical distribution comprises a region of Amazon located in Brazilian and Venezuelan territory. They are: Araraibo, Asurini, Baniwa, Deni, Fulniô, Guajajara, Jamamadi, Kaapor, Krahô, Kubeo, Kuikuro, Maku, Makuna, Pankararu, Pareci, Pataxó, Paumari, Tembé, Terena, Ticuna, Tiriyó, Tukano, Xokleng, Xukuru, Yanomami and Yawalapiti (Rodrigues et al. 2006), and Pataxós (Cunha et al. 2012). The study was conducted with five multiethinic indigenous communities at the municipality of São Gabriel da Cachoeira, Amazonas. The communities are called: Cunuri, Tapira Ponta, Ilha das Flores, Curicuriari, and São Jorge. People interviewed in these communities belong to 10 different ethnic groups: Tukano, Dessana, Baré, Tariano, Piratapuia, Arapaço, Baniwa, Hupda, Curripaco and Bara (Kffuri et al. 2016).

Until 2006, few ethnopharmacological surveys were conducted among the Brazilian Indians (Rodrigues et al. 2006). Upto the present, only two new studies have been found indicating plants used for the CNS by Brazilian indians (Kufuri et al. 2016, Cunha et al. 2012). In these studies, 66 plants with possible effects on the CNS were listed, of which 46 were indicated as antimalarial, without specifying which symptoms they were acting on, and 20 as analgesics, stimulants, for headaches and fever.

The 358 cited plants are used to treat complaints and/or different diseases that may act actively on the CNS. All uses for the 68 mentioned diseases were classified according to Rodrigues et al. (2006), in accordance with the possible effect/action; grouping for example the plants destined for different pains, such as for headache and earache, in the category "Analgesic". Thus, 12 categories were defined, according to the similarities between their effects expected on the CNS. Categories defined were: analgesics, anticonvulsants, anxiolytics, hallucinogens, head illnesses, hypnotics, memory enhancers, stimulants, to counteract fever, tonic and/or adaptogens, weight control, and others (without defined effect). Also, a new category was added for antimalarial plants, since it was not indicated for which specific symptoms they were used in the study. The categories and the number of plants indicated in each caterory are avaiable on Figure 4.1, where six of them (marked by an asterisk) appear to exert possible psychoactive effect/action: hallucinogens, anxiolytics, head illnesses, stimulants, hypnotics, and memory enhancers.

As examples, category "Analgesics" encompasses 21 different uses (arthritis, analgesics, body aches, chest pain, anus pain, muscle pain, backbone pain, pain in the foot's sole, pain in the ribs, ear pain, lower uterine pain, rheumatic pain, headache, toothache, pain, lower extremity pain, otitis, painful joints, kidney pain, back pain and heart pain), totaling 145 plants used to relieve all these kind of pains. Category "Fever" refers to three uses (fever, fever in children and fever with pain), with 120 indicated plants. Category "Tonics and/or Adaptogens" includes 15 uses (aphrodisiac, physical debility resulted from malaria combat, age or general illness, to combat tiredness, drowsiness and inability to concentrate, elderly people who have difficulty to understand instructions and physical degeneration, general debility, slow elderly, tonics, to purify and strengthen the body, to restore virility, strengthen those who are weak and are no longer interested in life because of age, tonic for the elderly, neuromuscular problems and sexual weakness), with a total of 40 species used. Thereby, the same species may have been cited for more than one use.

Considering all these classifications and the various uses and indications attributed to plants, we see that categories "Analgesics" and "Fever" were the ones with the highest number of plants indicated, 145 and 120 species, respectively. The great number of indications to category "Analgesics" may be related to the non-specificity of this group that includes all kinds of pain. However, category "Fever" may be related to the high occurrence of malaria in the Amazon region, which has a characteristic symptom of elevated body temperature, thus leaving this category within the most indicated. Another class that is evident related to the indiginous culture is the category "Hallucinogens". Many of the plants indicated to this section are used by Indians in practices of shamanism, which supposedly alter the shaman's perception, in order to facilitate contact with the spiritual world, to perform their rituals (Rodrigues et al. 2006). As shown by

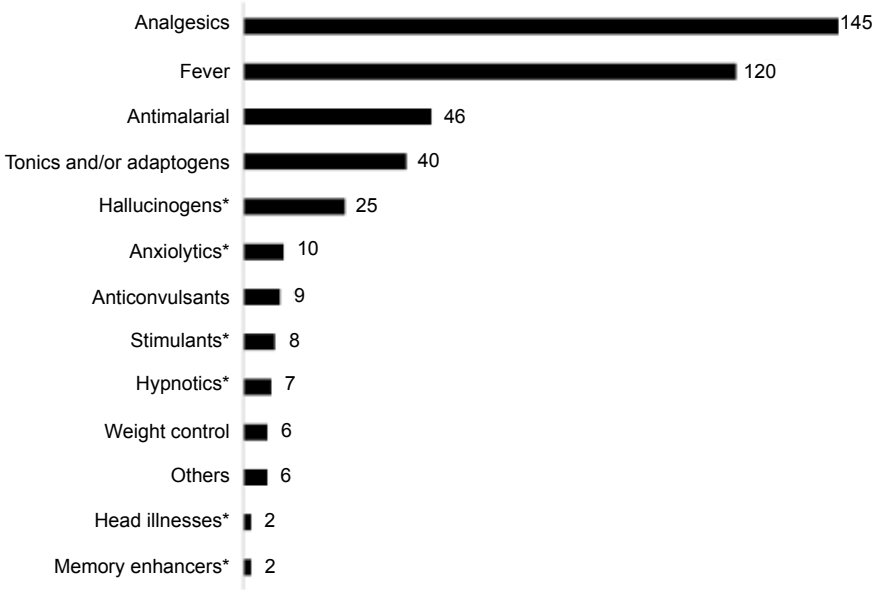


Fig. 4.1 Number of plants indicated, for each of the 13 categories, with possible effect on the CNS (adapted from Rodrigues et al. 2006).

Rodrigues et al. (2006), these plants' uses by Brazilian Indians may be directly related to their customs. The Krahô Indians, for example, were the ones who most indicated plants in the category "Tonics and/or Adaptogens" because they have the status of "champion runner" as extremely important for their culture, so, they use plants to be stronger and more able to win the competitions.

Among the plants phytochemically studied it was observed that seven phytochemical classes appeared more frequently: flavonoids (analgesia, anxiety, fever, hypnotic, stimulant, and weight control), alkaloids (head illnesses, hallucinogens, and as stimulant), essential oils (anxiety and fever), lignans (hallucinogens), tannins (anxiety), triterpenes and saponins (hypnotics). According to Rodrigues et al. (2006), these classes may be more common because they probably have a greater number of phytochemical constituents, which may contribute to Indians determining their uses. Table 4.1 shows the frequency of the 13 phytochemical classes among the plants indicated by Brazilian Indians, with possible effects on the Central Nervous System, together with the number of indicated species and their chemical constituents, which were found in the scientific literature until the year of 2006.

Table 4.1 Frequency of different phytochemical classes among the plants indicated by Brazilian indigenous people, with possible effects on the Central Nervous System until 2006 (adapted from Rodrigues et al. 2006).

Categories of use (number of uses cited in the literature)	**Number of species**	**Chemical constituents found in the scientific literature (number of plants that present the chemical constituent listed)**
1-Analgesics: (21 different indications) 1-arthritis (pain); 2-analgesic; 3-body ache; 4-chest pain; 5-pain in the anus; 6-muscle pain; 7-pain in the backbone; 8-pain in the sole of the foot; 9-pain in the ribs; 10-ear ache; 11-pain in the lower womb; 12-rheumatic pain; 13-headache; 14-toothache; 15-pain; 16-lower extremity pain; 17-otitis (pain); 18-painful joints, 19-kidney pain; 20-back pain; 21-heart pain.	145	flavonoids (28); alkaloids (18); essential oil (18); phenolic acids (9); triterpenoid (9); tannins (6); coumarin (5); terpenes (5); diterpenoids (4); steroids (3); glycosides (2); saponins (2); iridoids (2); sesquiterpene lactones (2); lactones (2); labdane diterpenes (2); diterpene galactoside (1); eudesmane acids (1); kava-pyrones (1); ketones (1); lignans (1); aliphatic compounds (1); polyacetylene compounds (1); sesquiterpene alcohols (1); lignan (1); aldehydes (1); anthocyanins (1); cardenolides (1); furanone (1).
2-Fever: (3 different indications) 1-fever; 2-fever (children); 3-fever with pain.	120	flavonoids (26); essential oil (22); triterpenoid (17); tannins (13); alkaloids (12); saponins (6); coumarins (5); phenolic acids (7); iridoids (4); steroids (5); anthraquinones (2); terpenes (2); polysaccharides (2); sesquiterpene (2); lactones (2); lignans (2); diterpenoids neocucurbitacins (1); furanone (1); furanocoumarin (1); kava-pyrones (1); ketones (1); labdane diterpenes (1); naphthopyrone derivative (1); phenylpropanoid (1); phorbolesters (1); podocarpane diterpenoids (1); sesquiterpenic lactones (1); xanthones (1); diterpenoids (1); fatty acids (1); alkylresorcinols (1); amides (1); chromenes (1).
3-Tonics and/or adaptogens: (15 different indications) 1-aphrodisiac; 2-combat physical debilitation resulting from malaria; 3-age or general infirmity; 4-to combat tiredness; 5-drowsiness and inability to concentrate; 6-elderly who suffer difficulty in understanding instructions and physical degeneration; 7-general debility; 8-old people who are slow; 9-tonic; 10-to purify and fortify the body; 11-re-establishing virility; 12-to strengthening those who are weak and who no longer are interested in life because of age; 13-tonic for the elderly; 14-neuromuscular problems; 15-sexual debility.	40	alkaloids (4); coumarins (2); triterpenoids (2); flavonoids (1); lignans (1); essential oils (1); clerodane diterpenes (1); xanthones (1); terpenes (1); labdane diterpenoids (1).
4-Hallucinogens: (7 different indications) 1-hallucinogen; 2-additive; 3-inebriating snuff; 4-narcotic; 5-psychoactive; 6-substitute for Nicotiana tabacum; 7-to see far (shamanism).	25	alkaloids (10); lignans (5); coumarin (3); phenolic acids (3); flavonoids (2); cardiac glycoside (2); steroids (2); diterpenes (1); triterpenoids (1); tannins (1); O-methoxylated-C-glycosylflavones lactones (1); neolignans (1); furanocoumarins (1).

Table 4.1 contd. ...

...Table 4.1 contd.

Categories of use (number of uses cited in the literature)	Number of species	Chemical constituents found in the scientific literature (number of plants that present the chemical constituent listed)
5-Anxiolytics: (3 different indications) 1-calmative; 2-irritability and crying in small children; 3-to calm.	10	flavonoids (4); essential oils (4); tannins (2); alkaloids (2); triterpenoids (2); saponins (1); sterols (1); naphtoquinones (1); iridoids (1); glycosides (1)
6-Anticonvulsants: (4 different indications) 1-seizures (children); 2-seizures; 3-periodic attacks of an epileptic-like nature; 4-dizziness and blurred/darkened vision (seizure).	9	essential oil (1).
7-Hypnotics: (4 different indications) 1-to induce sleep; 2-insomnia; 3-sedative; 4-elderly find difficult to sleep.	7	flavonoids (3); triterpenoids (2); saponins (2); phenolic acids (1); essential oils (1); tannins (1); steroids (1); alkaloids (1).
8-Stimulants: (1 different indications) 1-stimulant.	8	flavonoids (3); alkaloids (2); steroids (1); lignans (1); proanthocyanidins (1); essential oils (1); purine alkaloids (1); indole alkylamines (1).
9-Weight control: (4 different indications) 1-to fatten dogs; 2-to lose weight; 3-to stimulate appetite; 4-when they refuse to eat and lose appetite.	6	flavonoids (2); alkaloids (1).
10-Others: (3 different indications) 1-antidote against curare; 2-antidote against *Dioclea* spp.; 3-as stimulant for growth of breasts.	6	flavonoids (4); tannins (3), ginkgolides (1); alkyl and arylalkyl-1,3-diols (1); saponins (1); alcohols (1); aldehydes (1); terpenes (1); triterpene (1).
11-Head illnesses: (1 different indications) 1-Craziness.	2	curare alkaloids (1).
12-Memory enhancers: (2 different indications) 1-improve memory; 2-old people who are forgetful.	2	No studies found

Plants used by indigenous communities cited in the literature and pharmacology studies

Based on the review published by Rodrigues et al. (2006) and updating the data for 2017, we found 358 plant species with possible CNS-related activity. In order to verify which species have already been studied or related to studies, and which are unprecedented for future studies, the number of pharmacology studies were searched in the current literature that have already been conducted with these 358 species. The search was performed in Pubmed databases by species' name followed by the word "pharmacology". Figure 4.2 shows the number of publications found for species indicated by Brazilian indigenous people with possible effect on the CNS.

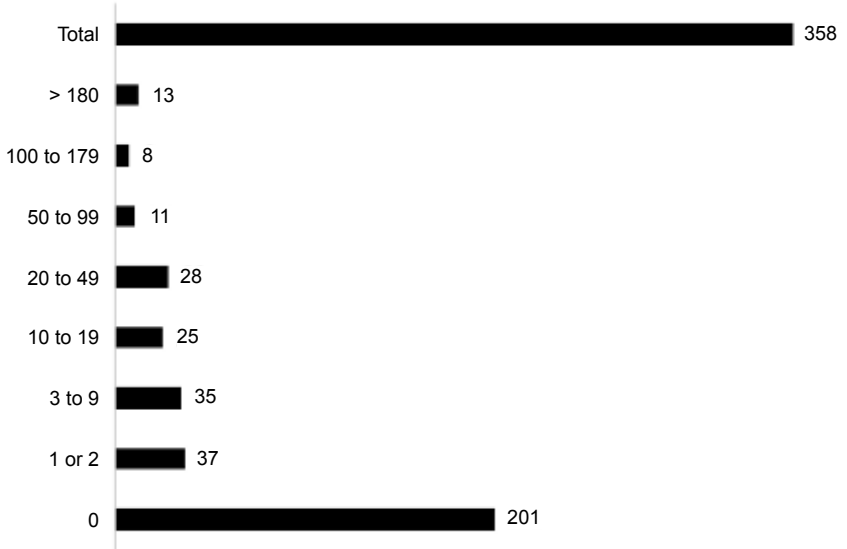


Fig. 4.2 Number of studies found (axis y) versus number of species (axis x) when searching for species' name followed by the word "pharmacology".

Table 4.2 An average of studies found in PubMed for each species with possible effect on the Central Nervous System.

Number of publications	Species with possible action on the Central Nervous System
More than 180 publications	*Aloe vera* (L.) Burm.f.*; Carica papaya* L.*; Citrus sinensis* (L.) Osbeck; *Cymbopogon citratus* (DC.) Stapf; *Cymbopogon citratus* Stapf.; *Helianthus annuus* L.; *Ipomoea batatas* (L.) Lam.; *Jatropha curcas* L.; *Melissa officinalis* L.; *Nicotiana tabacum* L.; *Ocimum basilicum* L.; *Ocimum gratissimum* L.; *Zingiber officinale* Roscoe.
Between 100 and 179 publications	*Ananas* sp.; *Carapa guianensis* Aubl.; *Cajanus cajan* (L.) Millsp.; *Mangifera indica* L.; *Mimosa pudica* L.; *Persea americana* Mill.; *Ruta graveolens* L.*; Terminalia catappa* L.
Between 50 and 99 publications	*Achillea millefolium* L.; *Argemone mexicana* L.; *Bixa orellana* L.; *Citrus aurantifolia* (Christm.); *Lactuca sativa* L.; *Lippia alba* L.; *Lippia alba* (Mill.) N.E. Br; *Musa paradisiaca* L.; *Passiflora edulis* Sims; *Paullinia cupana* Kunth; *Pimpinella anisum* L.
Between 20 and 49 publications	*Achyrocline satureioides* (Lam.) DC.; *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm.; *Anacardium occidentale* L.; *Artemisia vulgaris* L.; *Banisteriopsis caapi* (Spruce ex Griseb.) C.V. Morton; *Cocos nucifera* L.; *Copaifera langsdorffii* Desf.; *Casearia silvestris* Sw.; *Erithroxylum coca* Lam.; *Erythroxylum coca* L. var. Ipadu; *Eugenia uniflora* L.; *Gossypium barbadense* L.; *Jathropa gossypiifolia* C.; *Ocimum canum* Sims; *Petiveria alliacea* L.; *Pithecellobium dinizii* Ducke; *Physalis angulata* L.; *Plectranthus amboinicus* (Lour.); *Protium heptaphyllum* (Aubl.) March; *Psychotria viridis* Ruiz & Pav.; *Schinus terebinthifolius* Raddi; *Senna alata* (L.) Roxb.; *Senna obtusifolia* (L.) H.S. Irwin & Barneby; *Solanum americanum* Mill.; *Tagetes erecta* L.; *Terebinthifolius Raddi*; *Turnera ulmifolia* L.; *Uncaria guianensis* (Aubl.) J.F. Gmel.
Between 10 and 19 publications	*Acanthospermum hispidum* DC.; *Anadenanthera macrocarpa* (Benth.) Brenan; *Citrus* sp.; *Chondrodendron tomentosum* Ruiz & Pav.; *Cyperus articulatus* L.; *Dipteryx odorata* (Aubl.) Willd.; *Euphorbia prostrata* Aiton; *Hedychium coronarium* Koen.; *Hymenaea courbaril* L.; *Justicia pectoralis* Jacq.; *Kielmeyera coriacea* Mart. & Zucc.; *Lantana trifolia* L.; *Lutea* L.; *Mimosa tenuiflora* (Willd.) Poir.; Myrcia multiflora (Lam.) DC.; *Neurolaena lobata* (L.) R. Br. ex Cass; *Passiflora alata* Curtis; *Porophyllum ruderale* (Jacq.) Cass.; *Pterodon emarginatus* Vogel; *Ptychopetalum olacoides* Benth.; *Sambucus australis* Cham. & Schltdl.; *Swartzia* sp.; *Urena lobata* L.; *Waltheria indica* L.; *Zanthoxylum rhoifolium* Lam.
Between 3 and 9 publications	*Acca sellowiana* (O. Berg) Burret*; Ampelozizyphus amazonicus* Ducke; *Anadenanthera peregrina* (L.) Speg.; *Aniba canelilla* (Kunth); *Annona mucosa* Jacq.; *Asclepias curassavica* L.; *Baccharis uncinella* DC.; *Borreria verticillata* (L.) G. Mey.; *Canna indica* L.; *Conyza floribunda* Kunth; *Costus spiralis* (Jacq.) Roscoe; *Coutarea hexandra* (Jacq.) K. Schum.; *Euterpe precatoria* Mart.; *Genipa americana* L.; *Hedyosmum brasiliense* Miq.; *Himatanthus lancifolius* (Müll. Arg.) Woodson; *Leonotis nepetifolia* (L.) R. Br.; *Libidibia ferrea* (Mart. Ex Tul.) L.P.Queiroz; *Luffa operculata* (L.) Cogn.; *Mandevilla illustris* (Vell.); *Ocimum micranthum* Willd.; *Piper arboreum* Aubl.; *Piper* sp.; *Polypodium polypodioides* (L.) Watt; *Pothomorphe umbellata* (L.) Miq.; *Psidium quineense* Sw.; *Renealmia alpinia* (Rottb.) Maas; *Rubus brasiliensis* Mart.; *Schwenkia americana* L.; *Siparuna guianensis* Aubl.; *Spiranthera odoratissima* A. St.-Hil.; *Strychnos guianensis* (Aubl.) Mart.; *Tachia grandiflora* Maguire & Weaver; *Tachigalia paniculata* Aubl.; *Urera baccifera* (L.) Gaudich. ex Wedd.
1 or 2 publication(s)	*Alpinia nutans* Roscoe; *Andropogon leucostachyus* Kunth; *Annona hypoglauca* Mart.; *Arrabidaea brachypoda* (DC.) Bureau; *Aspidosperma nitidum* Benth. ex Müll. Arg.; *Astrocaryum aculeatum* G. Mey.; *Boerhavia coccinea* Mill.; *Cassia quinquangulata* Rich.; *Chaptalia nutans* (L.) Pol.; *Chelonanthus alatus* (Aubl.) Pulle; *Cordia trichotoma* (Vell.) Arrab. ex Steud.; *Crateva tapia* L.; *Cyathula prostrata* (L.) Blume Blume; *Desmodium incanum* DC.; *Derris floribunda* B. (Benth.) Ducke; *Diospyros guianensis* (Aubl.) Gürke.; *Epidendrum nocturnum* Jacq.; *Himatanthus bracteatus* (A. DC.) Woodson; *Laetia procera* (Poepp.) Eichler; *Licania heteromorpha* Benth.; *Lilium* sp.; *Ludwigia nervosa* (Poir.) H. Hara; *Miconia rubiginosa* (Bonpl.) DC.; *Montrichardia arborescens* (L.) Schott; *Omphalea diandra* L.; *Panopsis rubescens* (Pohl) Rusby; *Passiflora laurifolia* L.; *Parahancornia amapa* (Huber) Ducke; *Peperomia macrostachya* (Vahl) A Dietr.; *Phyllanthus acuminatus* Vahl; *Rolandra fruticosa* (L.) Kuntze; *Solanum asperum* Rich; *Solanum crinitum* Lam.; *Solanum mauritianum* Scop.; *Tanaecium nocturnum* (Barb.); *Virola calophylla* (Spruce) Warb.; *Wulffia baccata* (L.) Kuntze; *Zanthoxylum pentandrum* (Aubl.) R.A. Howard.

Table 4.2 contd. ...

...Table 4.2 contd.

Number of publications	Species with possible action on the Central Nervous System
no publications found	*Abuta concolor* Poepp. & Endl.; *Abuta imene* (Mart.) Eichler; *Abuta grisebachii* Triana & Planch.; *Abuta rufescens* Aubl.; *Adiantum serratodentatum* Humb. & Bonpl. ex Willd.; Alexa grandiflorsa Ducke; *Alsodeia guianensis* (Aubl.) Eichler.; *Alternanthera dentata* (Moench) Stuchlik ex R.E. Fr.; *Amasonia angustifolia* Mart. & Schauer; *Amasonia campestris* (Aubl.) Moldenke; *Anacardium giganteum* W. Hancock ex Engl.; *Aristolochia medicinalis* R.E. Schult.; *Arrabidaea trailii* Sprague; Aspidosperma discolor A DC.; *Aspidosperma schultesii* Woodson; *Asplenium formosum* Willd.; *Attalea maripa* (Aubl.) Mart.*; Axonopus pulcher* (Nees) Kuhlm.; *Baccharis cylindrica* (Less.) *DC.; Banara guianensis Aubl.; Bauhinia acreana* Harms; *Bertiera guianensis* Aubl.; *Brosimum acutifolium* Huber; *Brugmansia insignis* (Barb. Rodr.) R.E. Schult.; *Bulbostylis junciformis* (Kunth) C.B. Clarke; *Bulbostylis lanata* (Kunth) C.B. Clarke; *Calliandra tenuiflora* Benth; *Callichlamys latifolia* (Rich.) K. Schum.; *Canna* sp.*; Cassia latifolia* G. Mey.; *Centropogon surinamensis* (L.) C. Presl; *Cestrum laevigatum* Schltdl.; *Chondrodendron platiphyllum* (A. St.-Hil.) Miers; Cissus sulcicaulis (Baker) Planch.; *Clavija membranacea* Mez; *Clitoria guianensis* (Aubl.) Benth.; *Clitoria javitensis* (Kunth) Benth.; *Coccocypselum guianense* (Aubl.) K. Schum.; *Cochlospermum orinocense* (Kunth) Steud; *Cochlospermum regium* (Mart.); *Comolia microphylla* Benth.; *Complaya trilobata* (L.) Strother; *Connellia virginica* L.; *Conomorpha obovata* (Ruiz Lopez & Pavon) Werdermann; *Coussapoa intermedia* Miq.; *Coutoubea ramosa* Aubl.; *Crateva benthamii* Eichler; *Crepidospermum goudotianum* (Tul.) Triana & Planch.; *Crotalaria maypurensis* Kunth; *Curculigo scorzoneraefolia* (Lam.) Baker; *Cybianthus subspicatus* Benth. ex Miq.; *Cymbopogon densiflorus* (Steud.) Stapf.; *Cyperus flavus* J. Presl & C. Presl; *Declieuxia fruticosa* (Willd. ex Roem. & Schult.) Kuntze; *Deguelia amazonica* Killip*; Desmodium axilare* (Sw.) DC.; *Dialium guianense* (Aubl.) Sandwith; *Dichorisandra affinis* Mart.; *Dioclea elliptica* R.H. Maxwell; *Dioclea erecta* Hoehne; *Dioclea glabra* Benth.; *Dioclea latifolia* Benth.; *Dioclea scabra* (Rich.) R.H. Maxwell; *Dioclea ucayalina* Harms; *Diodia ocimifolia* (Willd. ex Roem. & Schult.) Bremek.; *Discolobium leptophyllum* Benth.; *Dorstenia asaroides* Hook.; *Duguetia duckei* R.E. Fr.; *Elionurus adustus* (Trin.) Ekman; *Elizabetha princeps* Schomburgk ex Benth.; *Eperua campestris* (Ducke) Ducke; *Eriochrysis cayennensis* P. Beauv.; *Erythrina glauca* Willd.; *Eugenia cauliflora* O. Berg; *Euterpe catinga* Wallace; *Ficus anthelmintica* Mart.; Ficus paraensis (Miq.); *Galipea jasminiflora* (A. St.-Hil.) Engl.; *Glycydendron amazonicum* Ducke*; Guatteria guianensis* (Aubl.) R.E.Fr.; *Guettarda viburnoides* Cham. & Schltdl.; *Gurania pachypoda* Harms; Helicostylis tomentosa (Poepp. & Endl.) Rusby; *Henriettea granulata* O. Berg & Triana; *Heteropsis tenuispadix* G.S.Bunting *; Humiriastrum piraparanense* Cuatrec.; *Hybanthus calceolaria* (L.) Schulze-Menz; *Hymenaea aurea* Y.T. Lee & Langenh.; *Hyptis hirsuta* Kunth; *Imperata brasiliensis* Trin.; *Ipomoea schomburgkii* Choisy; *Ipomoea wrighti* A.Gray; *Iriartea deltoidea* Ruiz &Pav; *Jacaranda copaia* (Aubl.) D. Don; *Justicia pectoralis* Jacq. var. stenophylla Leonard; *Kielmeyera rugosa* Choisy; *Licania humilis* Cham. & Schltdl.; *Lonchocarpus floribundus* Benth; *Macrolobium bifolium* (Aubl.) Pers.; *Macrolobium campestre* Huber; *Malachra capitata* (L.) L.; *Manihot salicifolia* Pohl; *Mansoa standleyi* (Steyerm.) A.H. Gentry; *Maprounea guianensis* Aubl.; *Maquira calophylla* (Poepp. & Endl.) C.C. Berg; *Maquira sclerophylla* (Ducke) C.C. Berg; *Marcgraviastrum elegans* de Roon; *Mauritia minor* Burret; *Memora flavida* (DC.) Bureau & K. Schum.; *Mesechites trifidus* (Jacq.) Müll. Arg.; *Miconia holosericea* (L.) DC; *Micropholis cyrtobotrya* (Mart. ex Miq.) Baill.; *Micropholis guyanensis* (A. DC.) Pierre; *Mikania divaricata* Poepp.; *Mimosa hostilis* (Mart.) Benth.; *Monopteryx uaucu* Spruce ex Benth.; *Mucuna altisima* (Jacq.) DC.; *Nectandra pisi* Miq.; *Ocotea aciphylla* (Nees) Mez; *Odontocarya tripetala* Diels; *Oncidium nanum* Lindl.; *Operculina alata* (Ham.) Urb.; *Ormosia discolor* Spruce ex Benth.; Ouratea castaneifolia (DC.) Engl.; *Palicourea coriacea* (Cham.) K. Schum; *Panicum cyanescens* Nees ex Trin.; *Panicum nervosum* Lam.; *Panopsis sessilifolia* (Rich.) Sandwith; *Paspalum serpentinum* Hochst. ex Steud.; *Passiflora costata* Mast.; *Pavonia rosa-campestris* A.St.-Hil; *Peperomia magnoliifolia* (Jacq.) A. Dietr.; *Peperomia obtusifolia* (L.) A. Dietr.; *Perama hirsuta* Aubl.; *Periandra pujalu* Emmerich & Senna; *Phanera splendens* (Kunth) Vaz; *Phaseolus linearis* Kunth; *Phenakospermum guianensis* (A. Rich.) Endl. exMiq.; *Phyllanthus dinizii* Huber; *Phyllanthus orbiculatus* Rich.; Pilocarpus pennatifolius Lem.; Piper arborea Aubl.; *Piper asimum* (Spr.) Angely; *Piper daguanum* C. DC.; *Piptocarpha rotundifolia* (Less.) Baker; *Piresia leptophylla* Soderstr.; *Pleurothallis rubens* Lindl.; *Polygala asperuloides* Kunth; Pouteria ucuqui Pires & R.E. Schult.; *Protium pallidum* Cuatrec.; *Protium paraense* Cuatrec.; *Pterocarpus michelii* Britton; *Randia armata* (Sw.) DC.; *Rhynchospora barbata* (Vahl) Kunth; *Rhynchospora nervosa* (Vahl) Boeck.; *Roupala obtusata* Klotzsch; *Ruellia aff. malacosperma* Greenm.; *Ruellia geminiflora* Kunth; *Sabicea amazonenses* Wernham; *Sagotia brachysepala* (Müll.Arg.) Secco; *Salvertia convallariodora* A. St.-Hil; *Schizaea pennula* Sw.; *Schizolobium amazonicum* Huber ex Ducke; *Schlegelia macrophylla* Ducke; *Schlegelia roseiflora* Ducke; *Schoenobiblus daphnoides* Mart.; *Sciadotenia pachnococca* Krukoff & Barneby; *Scleria hirtella* Sw.; *Sipanea pratensis* Aubl.; *Sloanea rufa* Planch. ex Benth.; *Smilax aequatorialis* (Griseb.) A. DC.; *Spondias mombin* L.; *Stachytarpheta sprucei* Moldenke; *Stachytarpheta straminea* Moldenke; *Struthanthus flexicaulis* Mart.; *Strychnos javariensis* Krukoff; *Swartzia argentea* Spruce ex Benth.; *Swartzia picta* Benth.; *Swartzia recurva* Poepp.; *Syngonanthus oblongus* (Körn.) Ruhland; *Tabebuia barbata* (E.Mey.) Sandwith; *Tabernaemontana heterophylla* Vahl; *Tabernaemontana sananho* Ruiz; *Tachigalia myrmecophila* Ducke; *Talisia cerasina* (Benth.) Radlk.; *Tephrosia senna* Kunth; *Tetrapteris methystica*; *Theobroma subincanum* Martius in Buchner; *Tococa formicaria* Mart.; *Tocoyena formosa* (Cham. & Schltdl.) K. Schum.; *Trachypogon plumosus* (Humb. & Bonpl. ex Willd.) Nees; Trichilia macrophylla Benth.; Trichilia tocacheana C. DC.; *Unxia camphorata* L.f.; *Verbena erinoides* Lam.; *Virola calophylloidea* Markgr.; *Virola michelii* Heckel; *Virola theiodora* (Spruce ex Benth.) Warb.; *Vismia tomentosa* Ruiz & Pav.; *Xylopia nitida* Dunal; *Zornia gemella* Vogel.

Of the 358 species sought, no study was found for 201. Such plants are indicated in Table 4.2, together with the number of studies found for each species. This result indicates that more than half of the vegetal species, indicated by Brazilian indigenous populations in the ethnopharmacological surveys, with therapeutic purpose and possible action on the CNS were not studied, which reveals a great study potential.

Conclusion

Brazil has a great endemic diversity of vegetal species, added to the numerous indigenous ethnic groups which use these plants as medicine for CNS disorders. Therefore, the use of these species by these cultures points out several bioactive potential that must be pharmacologically and phytochemically studied in order to develop new therapies and medicines for diseases recognized by biomedicine. However, for 176 species out of 358 reported in the literature, no pharmacological studies have been conducted to verify the efficacy and safety of its uses.

Still, few studies that list medicinal plant used by indigenous Brazilian describe their contexts of use, practices, beliefs involved in medico-religious treatments and the possible ways of choosing these plants by the curators. Thus, when conducted, many of pharmacological studies are erroneously interpreted as 'non-effective', since tests do not reproduce the circumstances in which species are used and do not embrace their cultural meanings.

Therefore, in order to successfully develop new medicines and therapeutic practices from vegetable species used by indigenous people, we must firstly understand, appreciate and admit this millenarian knowledge for later investigating the efficacy and safety of these species, considering the context, practices and values involved in their uses.

References

Albuquerque, U.P. 2005. Introdução à etnobotânica. Rio de Janeiro: Editora Interciência.

Albuquerque, U.P. and Lucena, R.F.P. 2005. Can apparency affect the use of plants by local people in tropical forests? Interciencia. 30: 506–511.

Alexiades, M.N. 1996. Selected Guidelines for Ethnobotanical Research: A Field Manual. New York: The New York Botanical Garden.

Balick, M.J. and Cox, P.A. 1996. Plants, people, and culture: the science of ethnobotany. Scientific American Library.

Bernard, H.R. 1988. Research methods in cultural anthropology. California: Sage Publications.

Cavalli-Sforza, L.L. and Feldman, M.W. 1981. Cultural transmission and evolution: a quantitative approach. Princeton University Press, Princeton, NJ.

Cechinel Filho, V. and Yunes, A.R. 1998. Estratégias para a obtenção de compostos farmacologicamente ativos a partir de plantas medicinais. Conceitos sobre modificação estrutural para otimização da atividade. Quim. Nova 21(1): 99–105.

Cunha Lima, S.T., Rodrigues, E.D., Alves, C., Merrigan, T.L., Melo, T., Guedes, M.L.S., Nascimento, A.F. and Toralles, M.B. 2012. The use of medicinal plants by an indigenous Pataxó community in NE Brazil. Rev. Bras. Plantas Med. 14(1): 84–91.

de Oliveira, F. C., Albuquerque, U. P., da Fonseca-Kruel, V.S. and Hanazaki, N. 2009. Avanços nas pesquisas etnobotânicas no Brasil. Acta Bot. Bras. 23(2): 590–605

de Medeiros, P.M., Ladio, A.H. and Abuquerque, U.P. 2015. Local criteria for medicinal plant selection. pp. 149–162. *In*: Albuquerque, U.P., de Medeiros, P.M. and Casas, A. (eds.). Evolutionary Ethnobiology. Springer International Publishing.

Elisabetsky, E. 2003. Etnofarmacologia. Ciênc. Cult. 55(3): 35–36.

FUNAI - Fundação Nacional do Índio. Índios no Brasil. 2016. Available at http://www.funai.gov.br/index.php/indios-no-brasil/quem-sao. Accessed on 10 Jun 2017.

Giulietti, A.M., Harley, R.M., Queiroz, L.P., Wanderley, M.G.L. and Van Den Berg, C. 2005. Biodiversidade e Conservação das Plantas no Brasil. Megadiversidade, Belo Horizonte 1(1): 52–61.

Gyllenhaal, C., Kadushin, M.R., Southavong, B., Sydara, K., Bouamanivong, S., Xaiveu, M. and Dac, L.X. 2012. Ethnobotanical approach versus random approach in the search for new bioactive compounds: support of a hypothesis. Pharm. Biol. 50(1): 30–41.

Heinrich, M., Ankli, A., Frei, B., Weimann, C. and Sticher, O. 1998. Medicinal plants in Mexico: Healers' consensus and cultural importance. Soc. Sci. Med. 47: 1859–1871.

Holmstedt, B. and Bruhn, J.G. 1983. Ethnopharmacology—a challenge. J. Ethnopharmacol. 8: 251–256.

IBGE - Instituto Brasileiro de Geografia e Estatística. Manual Técnico da Vegetação Brasileira. 2017. Available at: http://www.ibge.gov.br/home/geociencias/recursosnaturais/vegetacao/manual_vegetacao.shtm. Accessed on 04 Jun 2017.

International Society For Ethnopharmacology. Available at: www.ethnopharmacology.org/. Accessed on 10 Jun 2017.

ISA - Instituto Socioambiental. Povos Indígenas no Brasil. Available at: https://pib.socioambiental.org/pt/c/0/1/2/populacao-indigena-no-brasil. Accessed on 19 Jun 2017.

Júnior, W.S.F., Cruz, M.P., Vieira, F.J. and Albuquerque, U.P. 2015. An evolutionary perspective on the use of hallucinogens. pp. 185–197. *In*: Albuquerque, U.P., de Medeiros, P.M. and Casas, A. (eds.). Evolutionary Ethnobiology. Springer International Publishing.

Kffuri, C.W., Lopes, M.A., Ming, L.C., Odonne, G. and Kinupp, V.F. 2016. Antimalarial plants used by indigenous people of the Upper Rio Negro in Amazonas, Brazil. J. Ethnopharmacol. 178: 188–198.

Leonti, M. 2011. The future is written: Impact of scripts on the cognition, selection, knowledge and transmission of medicinal plant use and its implications for ethnobotany and ethnopharmacology. J. Ethnopharmacol. 134(3): 542–555.

Leonti, M., Fernando, R., Sticher, O. and Heinrich, M. 2003. Medicinal Flora of the Popoluca, Mexico: a Botanical Systematical Perspective. Econ. Bot. 57(2): 218–230.

Lewin, L. 1924. Phantastica: die betäubenden und erregenden Genussmittel.

Marcuse, H. 1973. A ideologia da sociedade industrial: o homem unidimensional. Giasone Rebuá's traduction. Rio de Janeiro: Zahar.

Melatti, J.C. 1978. Ritos de uma tribo timbira. São Paulo: Editora Ática.

Moerman, D. 2007. Agreement and meaning: Rethinking consensus analysis. J. Ethnopharmacol. 112: 451–460.

Pagani, E., Santos, J. de F.L. and Rodrigues, E. 2017. Culture-Bound Syndromes of a Brazilian Amazon Riverine population: Tentative correspondence between traditional and conventional medicine terms and possible ethnopharmacological implications. J. Ethnopharmacol. 203: 80–89.

Quinlan, M.B. 2010. Ethnomedicine and ethnobotany of fright, a Caribbean culture-bound psychiatric syndrome. J. Ethnobiol. Ethnomed. 6(1): 9.

Reyes-García, V. 2010. The relevance of traditional knowledge systems for ethnopharmacological research: theoretical and methodological contributions. J. Ethnobiol. Ethnomed. 6(1): 32.

Rodrigues, E. 2001. Usos rituais de plantas que indicam ações sobre o Sistema Nervoso Central pelos índios Krahô, com ênfase nas psicoativas. Doctoral dissertation, Universidade Federal de São Paulo. Escola Paulista de Medicina. Curso de Psicobiologia.

Rodrigues, E. and Carlini, E.A. 2005. Ritual use of plants with possible action on the central nervous system by the Krahô Indians, Brazil. PTR. Phytother. Res. 19: 129–135.

Rodrigues, E. and Carlini, E.A. 2006. A comparison of plants utilized in ritual healing by two Brazilian cultures: Quilombolas and Kraho Indians. J. Psychoactive Drugs 38: 285–295.

Rodrigues, E., Mendes, F.R. and Negri, G. 2006. Plants indicated by Brazilian Indians to Central Nervous System disturbances: a bibliographical approach. Curr. Med. Chem. 6: 211–244.

Rodrigues, E. and Otsuka, D.R. 2012. Estratégias utilizadas para a seleção de plantas com potencial bioativo com ênfase nos métodos da etnobotânica e etnofarmacologia. pp. 39–64. *In*: Carlini, E.A. and Mendes, F.R. 2011. Protocolos em psicofarmacologia comportamental: um guia para a pesquisa de drogas com ação sobre o SNC, com ênfase nas plantas medicinais. São Paulo: FAP-UNIFESP.

Scarano, F.R. 2009. Plant communities at the periphery of the Atlantic rain forest: rare-species bias and its risks for conservation. Biol. Conserv. 142(6): 1201–1208.

Schultes, R.E. 1988. Ethnopharmacological Conservation: A key to progress in medicine. Acta Amaz. 18: 393–406.

Schultes, R.E. and Hofmann, A. 1993. Plantas de los Dioses, orígenes del uso de los alucinógenos. Fondo de Cultura Económica.

Shephard, G.H. Jr. 2004. A sensory ecology of medicinal plant therapy in two amazonian societies. Am. Anthropol. 106: 252–266.

Soldati, G.T. and Albuquerque, U.P. 2012. Ethnobotany in intermedical spaces: the case of the Fulni-ô Indians (Northeastern Brazil). Evid.-Based Compl. Alt. 2012.

Svetaz, L., Zuljan, F., Derita, M., Petenatti, E., Tamayo, G., Cáceres, A., Cechinel Filho, V., Giménez, A., Pinzón, R., Zacchino, S.A. and Gupta, M. 2010. Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. J. Ethnopharmacol. 127: 137–158.

Tseng, W.S. 2006. From peculiar psychiatric disorders through culture-bound syndromes to culture-related specific syndromes. Transcult. Psychiatry 43(4): 554–576.

Waldstein, A. 2006. Mexican migrant ethnopharmacology: pharmacopoeia, classification of medicines and explanations of efficacy. J. Ethnopharmacol. 108(2): 299–310.

5

Ethnobotanical Retrospective and Features of the Multipurpose Plant *Genipa americana* L. (Rubiaceae)

Kyria Cilene de Andrade Bortoleti,[1] *Roberta Lane de Oliveira Silva,*[2] *Silvany de Sousa Araújo,*[2,3] *Ana Christina Brasileiro-Vidal*[2] and *Ana Maria Benko-Iseppon*[2,*]

Introduction

Genipa americana L. is an edible species of the Rubiaceae Juss. family, native to the northern part of South America occurring from the Caribbean region and southern Mexico to the Brazilian Atlantic coast and up to southern Peru. It is popularly called *genipap*, *jenipapo*, *huito*, or *jaguar*, among other names. This species was already used by the Incas (under the name of *wituq* or *hawa*) who used this plant for many purposes, with emphasis on body painting during rituals. It is generally a small tree (8–20 m), but representatives with up to 40 m have been described in Atlantic and Amazonian rainforests. Flowers are large (up to 15 cm diameter) round berries, with up to 400 g in weight pollinated by bees and flies. In addition to being edible and medicinal, the fruit is used for the production of dyes, syrup, sugar, as liqueurs, wine, ethanol and non-alcoholic beverages. Bark and resin are used in perfumery, pharmacy or for insecticide, fungicide, and antimicrobial or similar applications, fresh or dried, whether or not cut, powdered or crushed (Barbosa 2008, Codignoto et al. 2017). The wood is a source of tannins and their salts, esters, ethers are used for the production of natural blue and black dyes. The flowers are used in the manufacture of essential oils for the perfumery industry (Borges and Rezende 2000), and the leaves are used for feeding animals.

This species is rarely cultivated, being thus exploited through extractive practices, i.e., plant material is collected from natural populations and no traditional cultivation exists. Such practices have contributed to some loss of diversity due to deforestation, forest fragmentation and some reproductive problems due to the genetic features of this species.

Most of the existing ethnobotanical bibliography about *G. americana* is published in Portuguese or Spanish, thus being unavailable to the wide scientific community. The present chapter describes the

[1] UNIVASF, Universidade Federal do Vale do São Francisco, Petrolina, Pernambuco, Brazil.
[2] UFPE, Universidade Federal de Pernambuco, Genetics Department, Center of Biosciences, Av. Prof. Moraes Rego, 1235, 50.670-423, Recife, Pernambuco, Brazil.
[3] UFRPE, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil.
* Corresponding author: ana.benko.iseppon@pq.cnpq.br

traditional use of this species by human populations. It also highlights available studies of this medicinal and edible plant, as well as implications associated with genetic, environmental, and market potential for derivatives from this interesting species.

Taxonomy and morphological features of *G. americana* L.

The genus *Genipa* L. is classified in the tribe Gardenieae, subfamily Ixoroideae of the family Rubiaceae Juss. (Barroso et al. 1991). Gentry (1993) described six distinct species of the genus. Later, Pierozzi and Mendaçolli (1997) recognized only three species (*G. americana, G. infundibuliformis* Zappi & Semir, and *G. williamsii* Standl.). However, based on phylogenetic studies, Persson (2000a, 2003) replaced *G. williamsii* in the genus *Agouticarpa* [*Agouticarpa williamsii* (Standl.) C.H. Perss.]. Therefore, the genus *Genipa* includes only two species, being *G. americana* the most dispersed. Among the species cited, *G. americana* (Fig. 5.1) is widely distributed in tropical America and is considered the most known tree species of the genus (Guerra 1993, Zappi et al. 1995). In an inventory of the Amazonian angiosperms, Gentry (1993) highlighted the participation of the genus as a component of the Amazonian canopy, presenting fleshy and indehiscent fruits (Fig. 5.1a) with many seeds, which are dispersed by mammals. Its leaves are broad (Fig. 5.1e) with triangular subfoliar stipules. The color of the corolla varies from cream to yellow, with a tube approximately 1 cm wide and anthers inserted between the bases of the corolla lobes (Fig. 5.1b).

G. americana stands out as a woody dicotyledonous plant with high ecological plasticity, reaching up to 40 m in height. The upright trunk is 40 to 60 cm in diameter and its crown is branched and quite leafy, with an abundant verticillate branching (Fig. 5.1c), and bark is usually glabrous, smooth, thick and colored grayish-green with gray spots (Corrêa 1984, Figueiredo et al. 1986, Silva et al. 1998). Its leaves are simple, opposite, densified at the extremities of the branches, with triangular, sub-coriaceous, oblong-obovate, triangular-shaped, glabrous, and with irregularly reticulated secondary veins. It presents hermaphrodite flowers, axillary or terminal, slightly aromatic, tubular-campanulate calyx, yellow-white corolla (Figs. 5.1b, 5.2a), and bilocular ovary (Corrêa 1984, Figueiredo et al. 1986, Silva et al. 1998). The fruit is an ovoid

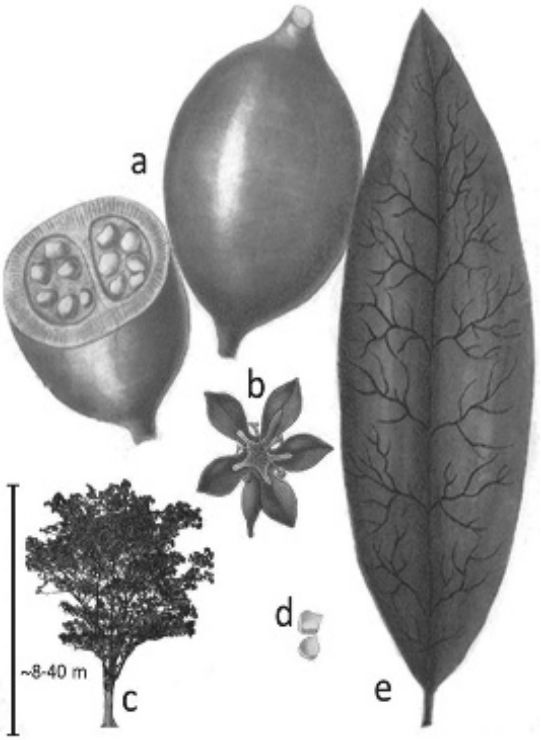


Fig. 5.1 Botanical illustration of *Genipa americana*. (a) fruit; (b) flower; (c) habit; (d) seeds and (e) leaf with nervures. Source: a-b/d-e from Johann Wilhelm Weinmann: *Phytanthoza iconographia*, Augsburg 1735–1745 (public domain). c. present work.

berry (Fig. 5.1a) of brown color, 5 to 12 cm in diameter, with a soft bark, thin and wrinkled membrane. It presents a delicate pulp of vinous dark color, juicy, aromatic and edible.

The seeds are located in the central portion of the fruit (Fig. 5.1a), being light-brown, flat and polished (Fig. 5.1d), surrounded by a colored aril, with viability of up to 90 days after fruit removal and zoochoric or hydrochoric dispersion (Braga 1960, Figueiredo et al. 1986, Silva et al. 1998). Seed germination is epigynous, that is, cotyledons rise above the soil surface. According to Nascimento et al. (2000), genipap seeds germinate more efficiently on a substrate with filter paper or vermiculite at a temperature of 30°C. Despite the massive production and wide seed dispersal, there is a high mortality rate after germination. Therefore, rare seedlings of this species are found in advanced stages in the natural habitat (Francis 1993, present work).

This species also exhibits peculiar reproductive mechanisms. Salomão and Allem (2001) reported that *G. americana* would present apomictic reproduction and polyembryony. In apomixis, meiosis and fertilization are altered, with seed formation without fertilization, that is, the offspring receives genetic material from a parent, while the other gametes, usually male, stimulate the division of the zygote. In turn, polyembryony is the formation of more than one embryo per egg. In their experiments, Salomão and Allem (2001) observed that *G. americana* seedlings from polyembryony had a uniform development.

Origin and geographical distribution of *G. americana*

There are controversies as to the origin of *G. americana*. Based on a bibliographical survey, Xavier (1976) suggested that this plant originated from humid areas of Cuba and Puerto Rico, while other researchers believe that the center of origin of genipap is tropical America. According to Cavalcante (1991), the American *Genipa* species originated from northern South America, where it is found both in the wild and cultivated forms, since pre-Columbian times. However, it may be emphasized that this situation applies to several areas of current occurrence of the species, for example, the Brazilian Atlantic coast till the state of Bahia, in Brazil. Considering that the Amazonian region hosts the greatest diversity of *Genipa* species, it can be considered that this is the most probable region of origin of the genus and probably of *G. americana*.

Due to the lack of fossil records, the original limits of *Genipa* distribution are unknown. Currently, the natural distribution of the species is restricted to areas with annual rainfall of 1200 to 4000 mm and average annual temperature between 18 and 28°C (Francis 1993). According to Sebbenn et al. (1998), genipap is distributed naturally between latitudes 20 °N (Mexico) to 20 °S (Brazil, SP). Its culture extends throughout the South American continent, Central America and the Antilles, being common in all the Brazilian Amazon, in the natural and cultivated state. The expressive dispersion of this species throughout America must be attributed to its being one of the checked main plants cultivated by the Indians in pre-Columbian times (Francis 1993). This use was reported in several letters sent from Brazil to Europe in the 16th century (Cavalcante 1991).

In Brazil, *G. americana* occurs in semi-deciduous seasonal forests, with greater abundance in wetlands and riverbanks, supporting long-lasting floods, being also found in dry lands of the slopes or colonizing open areas, behaving as a secondary pioneer (Andrade et al. 2000, Lorenzi 2002).

Phylogenetic studies on *Genipa* and *G. americana*

Phylogenetic analyses are determinant for ecological, evolutionary and biogeographic inferences, as well as for morphological and anatomical investigations of different species (Bremer 2009). With the advancement of genetic sequencing techniques, the growing generation of nuclear and organellar DNA sequences associated with the morphological characteristics has provided valuable data, which supports the phylogenetic evaluation of plant species at different taxonomic levels.

The genus *Genipa* is grouped in the tribe Gardenieae, subfamily Ixoroideae that is designated as monophyletic (Kainulainen et al. 2013) and diverged to 73.1 Mya (million years ago) within the Rubiaceae (Bremer and Eriksson 2009). In order to determine the phylogeny and to evaluate the tribal delimitations within Ixoroideae, Kainulainen et al. (2013) used information from six coding (*mat*K, *ndh*F, *rbc*L) and non-coding plastid DNA regions (the rps16 intron, *trnS*-G and *trnT*-F) obtained for 110 species, representative

of 87 genera, for phylogenetic reconstructions using Bayesian analysis and Maximum Parsimony. These authors concluded that the Ixoroideae was composed of three groups, among them the Coffeeae-alliance, which involves the Gardenieae tribe (including *Genipa*), designated as the largest of the subfamily (Bremer and Eriksson 2009). Different morphological and molecular evaluations point the Gardenieae as a polyphyletic group (Bremer 2009, Bremer and Eriksson 2009, Kainulainen et al. 2013, Mouly et al. 2014), being recognized as an unresolved group, both in terms of intertribal relations and in tribal boundaries.

Originally, the Gardenieae tribe was described by de Candolle (1830) and was constituted by 28 genera whose representatives were characterized by the presence of biloculated ovaries and indiscriminate fruits with many seeds, like *Genipa*. Later, also using morphological data, Robbrecht and Puff (1986) recognized two subtribes (1) Diplosporinae and (2) Gardeniinae, composed of 20 and 60 genera, respectively. In the Gardeniinae subtribe, three groups were recognized (1) Tetrad-, (2) Aidia and (3) Alibertia, the latter being composed of six Neotropical genera including *Genipa*. With the addition of plasmidial DNA data to the morphological matrix, a later phylogeny proposed by Persson (1996) supported the three groups mentioned. However, it suggested the exclusion of *G. americana* from the Alibertia group, based on data for *rps16* intron and the intergenic spacer *trnL*-F, confirming the proposed by Andreasen and Bremer (1996, 1997) with morphological and molecular data such as *Rbc*L gene. Another phylogenetic study with 38 species from the Alibertia group using the untranscribed spacer of 5S rDNA and Internal Transcribed Spacers (ITS) revealed a clustering of *Genipa* aff. *willianssi* in a basal clade of the group Alibertia. In turn, *G. americana* was grouped with the paleotropical group. According to the authors, the plastid DNA data indicate that the *Genipa* genus is paraphyletic (Persson 1996, Persson 2000), a fact that needs to be evaluated with other phylogenetically informative sequences.

Up to date, no infrageneric evaluation was carried out using molecular markers in the genus *Genipa*. Therefore, important questions, such as the number of species, monophyly, and infrageneric relations remain unresolved.

Cytogenetic studies

Previous reports on mitotic and meiotic chromosomes of *G. americana* have revealed very unusual karyotypic features for this species. The chromosome number $2n = 2x = 22$ was reported for *G. americana* by Gibbs and Ingram (1982) and was confirmed by several other authors (Guerra 1986, Kiehn 1986, Pierozzi and Da Cruz 1988, Guerra 1993, Pierozzi and Mendaçolli 1997, Corrêa and Forni-Martins 2004) and also the present work (Fig. 5.2b), being recognized as a common number in the family Rubiaceae.

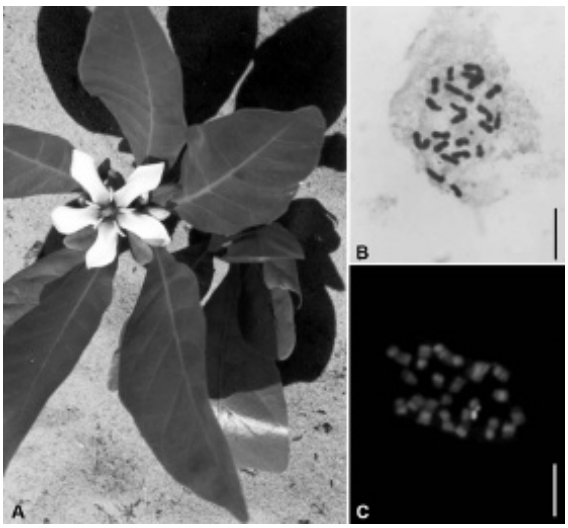


Fig. 5.2 *Genipa americana.* (a) Detail of a branch with a flower, flower buds and leaves, collected in Pernambuco, Brazil. (b) Mitotic chromosomes ($2n = 22$) after standard staining with Giemsa, and (c) after CMA_3 (chromomycin A_3) fluorochrome staining. Bars in (b) and (c) = 10 μm.

Diploid genipap individuals present an asymmetric karyotype with the presence of four to seven metacentric and submetacentric chromosomes, respectively, with a chromosome size of 2.42 to 6.77 μm (Corrêa and Forni-Martins 2004). These data, except the chromosome count, diverged from that observed by Pierozzi and Mendaçolli (1997) who suggested smaller chromosomes and the occurrence of three and eight metacentric and submetacentric chromosomes, respectively.

Also regarding the heterochromatic bands evidenced by C-banding, some divergences among distinct studies are evident. Pierozzi and Da Cruz (1988) emphasized centromeric and telomeric heterochromatic blocks in the genipap chromosomal complement, while Pierozzi and Mendaçolli (1997) showed the predominance of pericentromeric bands. A preliminary evaluation carried out by our group indicated some variation in the distribution of heterochromatic bands in populations collected in Pernambuco (Brazil).

A hallmark feature for *G. americana* was revealed by staining with the CMA_3/DAPI (4'-6'-diamidino-2-phenylindole) base-specific fluorochromes. A high amount of constitutive GC-rich heterochromatin (Guanine-Cytosine) was evidenced by the CMA_3^+ (CMA_3 positive) bands pattern (Guerra 1993). In some chromosomes, these heterochromatic segments constituted about 50% of their extent, as can be observed in Fig. 5.2c (present work), which makes *G. americana* the plant species with the highest known amount of heterochromatin in tropical America (Guerra 2001).

We presume that such an intraspecific variation and the large amounts of satellite repetitive DNA may result in meiotic instabilities, leading to the generation of unbalanced gametes, which could justify the reports of low seed germination rate fertility for the species. This could be in consonance with the proposed occurrence of apomixis as a reproductive strategy of *G. americana*, as suggested by Salomão and Allem (2001). To clarify this question, additional meiotic studies should be carried out as well as population studies.

Molecular diversity

Studies aimed at acquiring knowledge about the genetic diversity of a particular native species provide valuable information for the improvement of *ex situ* and *in situ* conservation strategies, especially concerning species considered vulnerable due to the reduction of genetic diversity (Sebbenn et al. 1998). This is the case of *G. americana*, which has been affected by extractive practices and has also been used for the recovery of degraded areas, mainly in the restoration of riparian forests (Sebbenn et al. 1998, Rabbani et al. 2012).

Molecular studies with genipap are still scarce and involve the use of different markers, including isoenzymes (Sebbenn et al. 1998, Sebbenn et al. 2003, Sebbenn 2004), RAPD (Random Amplified Polymorphic DNA; Rabbani et al. 2012), ISSR (Inter Simple Sequence Repeats; Santos 2012, Moura 2014, Silva et al. 2014) and SSRs (Simple Sequence Repeats; Manoel 2014, Manoel et al. 2014, Manoel et al. 2015). Existing reports with molecular markers aimed at obtaining information about genetic structure and diversity, breeding system and gene flow of the species in question, for the purposes of forest recovery strategies, conservation, management and breeding programs (Rabanni et al. 2012, Santos 2012).

The isoenzyme electrophoresis was used to evaluate the genetic variability, reproductive system and spatial distribution of a natural population of *G. americana* located in the riparian forest of the Mogi Guaçu River (São Paulo, Brazil). The evaluation included 42 adult plants and 300 seedlings from 15 matrices (20 seedlings per matrix). The evaluated isoenzymatic loci were: PGM (Phosphoglucose), 6PGDH (6-phosphogluconate dehydrogenase), PGI (Phosphoglucose isomerase), MDH (Malate dehydrogenase) and PRX (Peroxidase). Polymorphism levels indicated that this population presented some potential for exploration in future breeding programs and for seed collection for the purpose of recovery of degraded areas (Sebbenn et al. 1998, Sebbenn et al. 2003). Additionally, the authors evidenced an excess of heterozygotes in adult plants, as compared with seedlings, suggesting the existence of selection in favor of the heterozygotes between the adult plants in different development stages. On the other hand, an excess of homozygotes was noticed among the seedlings, indicating the occurrence of inbreeding, leading to deviations from the Hardy-Weinberg equilibrium, raising the assumption that the population would be reproductively subdivided into two groups with a certain degree of relatedness, possibly due to preferential crosses and/or genetic drift. Finally, the estimate of the crossing rate of the population in question showed that 81.6% of the *G. americana* seedlings were generated by crossing, being 61.7% random, 19.9% between related and 18.4% regarded possible apomictic (Sebbenn et al. 1998).

Later, Sebbenn (2004) studied the inheritance and linkage disequilibrium in four polymorphic isoenzymatic loci (*6pghd-1*, *Pgi-2*, *Mdh-1* and *Mdh-2*) in a population of *G. americana*. Segregation was homogeneous among individuals, regarding two to three alleles. However, significant deviations of the 1:1 expected ratio were detected in the *Mdh-2* and *6pghd-1* whose causes may be selection, meiotic distortion, inter-allelic interactions and gene drift. Signs of linkage disequilibrium were not detected for any of the loci, so the author suggested that they can be used for studying the breeding system, diversity and genetic structure of populations of *G. americana* (Sebbenn 2004).

RAPD molecular markers were used to evaluate the genetic diversity of two natural populations located along the lower São Francisco River (Rabanni et al. 2012) and in Arauá (Silva et al. 2015) in the state of Sergipe (Brazil). In both studies, a high level of polymorphism among the evaluated individuals was noticed, emphasizing that these markers regard useful tools to infer about the population genetic diversity. Rabanni et al. (2012) also correlated the average genetic distances with the increase in geographic distances, pointing out that studied genipap populations would be differentiated by a random process, suffering the effects of genetic drift due to fragmentation and decrease of gene flow (Telles and Bastos 2009). Also, UPGMA (Unweighted Pair Group Method using Arithmetic Averages) analyses allowed the identification of adequate individuals to be used as seed matrices for germplasm bank generation, seedling production and restoration programs in degraded areas (Rabanni et al. 2012).

Another approach identified 32 microsatellite loci used to characterize 40 adult *G. americana* trees from two populations located in a small forest fragment at the Mogi Guaçu Ecological Station (São Paulo, Brazil) and in Selvíria (Mato Grosso, Brazil). Most of the loci identified regarded dinucleotides (80.49%), followed by mono- (10.97%), tetra- (4.88%), tri- (2.44%) and pentanucleotides (1.22%). Of these, 17 were polymorphic and 15 monomorphic (Manoel et al. 2014). This predominance of dinucleotides was also highlighted in genipap DNA sequences deposited in the public GenBank database (www.ncbi.nlm.nih.gov/genbank/) by Camargo et al. (2016). In terms of genetic structure and diversity, the number of alleles, the expected and observed heterozygosity for the populations of Mogi Guaçu and Selvíria showed that all loci were in Hardy-Weinberg disequilibrium (Manoel et al. 2014). This scenario is probably related to the occurrence of sampled individuals in small forest fragments. Among the 32 microsatellite loci described by Manoel et al. (2014), six were investigated in 188 adult and 163 regenerative trees of the Mogi Guaçu population, showing Mendelian inheritance, absence of genetic linkage and genotypic equilibrium, being indicated for studies of genetic structure and diversity, mating systems and pedigree analyses in this species (Manoel et al. 2015).

22 ISSR markers have also been used to evaluate genetic diversity in 35 accessions of genipap (Santos 2012, Moura 2014, Silva et al. 2014) in an experimental field in Cruz das Almas (state of Bahia, Brazil), revealing 86% polymorphism in the evaluated germplasm (Santos 2012). Another study accessed the diversity, divergence and spatial genetic structure of 76 individuals collected in six regions of the Sergipe state with 14 ISSR primers, among which seven generated 107 fragments with 100% polymorphism. The analysis of similarity and spatial genetic structure revealed the distribution of the individuals in three distinct groups, independent of the region of their origin, indicating that the genetic basis was distributed randomly (Moura 2014).

The Brazilian genipap germplasm bank was created in 2009 at Embrapa (Empresa Brasileira de Pesquisa Agropecuária) Coastal Tablelands station (Tabuleiros Costeiros, Sergipe). This germplasm bank was sampled using 12 ISSR primers, uncovering a higher diversity within the accessions (57%) than between (43%), indicating a strong genetic structure and a low gene flow. UPGMA phenogram showed the formation of four groups: C1, constituted mostly by accessions from Sergipe and Bahia; C2, accessions from Ceará and Sergipe; C3 formed by materials collected in Sergipe and Ceará, and C4, constituted by a single genotype of Sergipe. The authors suggest that this genetic distance may indicate a strong differentiation process, as a consequence of the genetic erosion, due to the current fragmentation of natural populations. Finally, the low heterozygosity noted among the evaluated genotypes indicates a low diversity in the genipap germplasm bank, suggesting the need to introduce new individuals to incorporate new alleles and increase genetic diversity (Silva et al. 2014).

Folk medicinal applications

South American populations still have a significant dependence on natural resources, with emphasis on plants for various purposes, including food and phytomedicines (Benko-Iseppon and Crovella 2010). In this scenario Rubiaceae members stand out, exhibiting a variety of chemical constituents of medicinal importance, being used in the treatment of bronchitis, asthma, pneumonia, as well as anti-rheumatic, emetic, purgative, diuretic, anti-inflammatory, antiviral and anti-edeogenic activity, among others (Lorenzi and Matos 2002, Dias et al. 2013). Within the family, genipap occupies a prominent position due to its recognized popular use, with some pharmacological studies that have confirmed part of its properties, also uncovering new therapeutic potentialities.

According to Cordeiro and Felix (2014), *G. americana* is among the most cited plant species by the population of Paraíba state (Northeastern Brazil), highlighting the use of genipap fruits in folk medicine for the treatment of osteoporosis, anemia, stomach problems, nervousness, diabetes, cholesterol, besides being an excellent tonic in the fight against indisposition, fatigue and weakness.

A compilation of the main uses of *G. americana* reported in the literature is shown in Table 5.1, involving mostly indications based on the application of interviews and questionnaires in populations in their areas of occurrence. All parts of the plants have medicinal activity (Table 5.1, Fig. 5.3a). For example, tea prepared from the roots has purgative and antigonorrheic properties. Infusion of the leaves is used for the treatment of diarrhea and syphilis, and when macerated it is also employed by some native tribes as antifebrile.

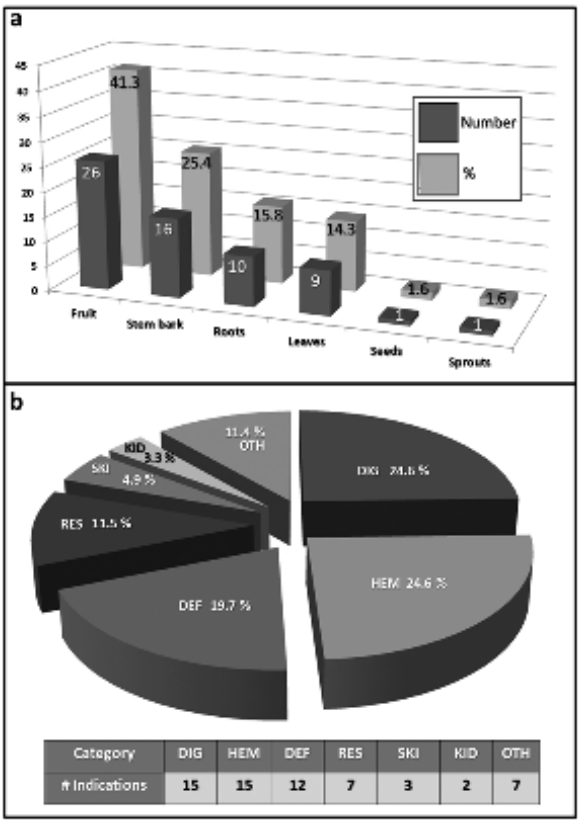


Fig. 5.3 (a) Most used *Genipa americana* plant parts based on Table 5.1, including the number of citations (dark gray) and percentage of citations (light gray). (b) Graphical representation of the main categories of indication (in percentage) based on the number of indications. Legend for abbreviations in b: DIG = digestive tract; HEM = hematological disturbs (e.g., blood pressure, stimulating the lymphatic system); DEF = defense against pathogens, and parasites, antimicrobial activity; RES = respiratory tract; SKI = skin, and muscles; KID = kidney and urinary tract; OTH = whole body, against fever, and nervousness, among other indications.

Table 5.1 Main ethnobotanical and phytotherapeutic indications of *G. americana* considering literature reports and considering no association with other plant species.

Ethnobotanical indication (or treatment of)	Plant part used	Form of use	Category[1]	Reference(s)
Against fever	Leaves	Maceration	OTH	Delprete et al. 2005
Against weakness	Fruit	Juice	OTH	Epstein 2001, Cordeiro and Felix 2014
Anemia	Fruit	Tonic	HEM	Agra et al. 2007
Anemia	Stem Bark	Decoction	HEM	Corrêa 1984, Donadio et al. 1998, Matos 1999, Cordeiro and Felix 2014
Anthelmintic	Leaves	Aqueous extract	DEF	Nogueira et al. 2014
Antiasthmatic	Fruit	*In natura*	RES	Mors et al. 2000, Epstein 2001, Moreira et al. 2002
Anti-diarrheic	Leaves	Tea	DIG	Corrêa 1984, Epstein 2001
Antigonorrheic	Roots	Tea	DEF	Mors et al. 2000, Epstein 2001, Lorenzi and Matos 2002, Agra et al. 2007, Alves et al. 2008
Anti-inflammatory	Leaves	Maceration	OTH	Delprete et al. 2005
Antimicrobial	Fruit	*In natura*	DEF	Barbosa 2008, Codignoto et al. 2017
Anti-syphilitic	Leaves	Infusion	DEF	Corrêa 1984, Epstein 2001
Blood depurative	Leaves	Decoction	HEM	Alves et al. 2008
Blood depurative	Stem bark	Decoction	HEM	Alves et al. 2008
Cholesterol decrease	Fruit	*In natura*	HEM	Cordeiro and Felix 2014
Diabetes	Fruit	*In natura*	OTH	Cordeiro and Felix 2014
Diuretic	Fruit	Juice	KID	Epstein 2001
Diuretic	Stem bark	N.I.[2]	KID	Donadio et al. 1998
Granular pharyngitis	Stem bark	Decoction	RES	Corrêa 1984, Matos 1999
Hypertension	Fruit	*In natura*	HIM	Robineau 1995
Jaundice	Fruit	*In natura*	HEM	Mors et al. 2000, Epstein 2001, Moreira et al. 2002
Liver disorders	Leaves	Infusion	DIG	Agra et al. 2007
Liver disorders	Fruit	*In natura*	DIG	Mors et al. 2000, Epstein 2001, Moreira et al. 2002
Muscle contusion, luxation	Stem bark	Decoction	SKI	Corrêa 1984, Matos 1999
Nervousness (calming)	Fruit	*In natura*	OTH	Cordeiro and Felix 2014
Osteoporosis	Fruit	*In natura*	OTH	Cordeiro and Felix 2014
Purgative	Roots	Tea	DIG	Mors et al. 2000, Epstein 2001, Lorenzi and Matos 2002, Agra et al. 2007, Alves et al. 2008
Purgative	Stem bark	N.I.[2]	DIG	Corrêa 1984
Respiratory clearance	Sprouts	N.I.[2]	RES	Epstein 2001
Respiratory clearance	Fruit	Juice	RES	Epstein 2001
Scurvy	Stem bark	Decoction	HEM	Corrêa 1984, Matos 1999
Skin affections	Fruit	Juice	SKI	Djerassi et al. 1961
Spleen disorders	Fruit	*In natura*	HEM	Mors et al. 2000, Epstein 2001, Moreira et al. 2002
Stomach pain	Fruit	*In natura*	DIG	Cordeiro and Felix 2014
Stomach ulcer	Stem bark	N.I.[2]	DIG	Donadio et al. 1998
Venereal ulcers	Stem bark	Decoction	DEF	Corrêa 1984, Matos 1999
Vomitory	Seeds	N.I.[2]	DIG	Epstein 2001

[1]Category of indications: DEF = defense against pathogens, and parasites, antimicrobial; DIG = digestive tract; HEM = hematological disturbs, blood pressure and lymphatic system; KID = kidney and urinary tract; RES = respiratory tract; SKI = skin, and muscles; OTH = whole body, against fever, nervousness, bones, etc. [2]N.I. = Not informed.

The stem and the bark, although having tannins, exhibit a predominantly purgative effect which in decoction, is indicated for the treatment of scurvy wound, venereal ulcer, granular pharyngitis, and anemia, besides being used in contusions and bone displacement.

Mature fruits are rich in iron and are indicated for problems like anemia, jaundice, asthma, liver and spleen affections, and against osteoporosis, diabetes and high cholesterol. In addition, due to its high content of mannitol, the mature fruit is recommended for the treatment of hypertension (Corrêa 1984, Matos 1999, Mors et al. 2000, Lorenzi and Matos 2002, Moreira et al. 2002, Delprete et al. 2005, Conceição et al. 2011). *In vitro* studies with extracts of the fruits of *G. americana* showed a broad spectrum antimicrobial activity (Barbosa 2008, Codignoto et al. 2017).

Considering the popular indications sampled, it is observed that the most used part involves the fruit (41.3%), generally used *in natura*, including both mature and unripe stages. The second most used part is the stem/bark (25.4%), followed by root (15.8%) and leaves (14.3%), while seeds and sprouts are rarely cited, and no phytotherapeutic use of the flowers was reported (Fig. 5.3a).

An evaluation of the categories of use or indication based on Table 5.1 is presented in Fig. 5.3b. Two categories figure among most indicated: DIG (diseases of the digestive tract) and HEM (treatment of blood and circulation problems, hematological disturbs, high blood pressure and disorders of the lymphatic system), both with 24.6%. The third category with 19.7% regarded defense (DEF, e.g., against pathogens, and parasites or antimicrobial effect), followed by affections of the respiratory system (RES, 11.5%). Less frequent indications included problems with skin and muscles (SKI = 4.9%), kidney and urinary tract (KID = 3.3%). All remaining symptoms were placed in the "other" (OTH = 11.4%) category, including problems that affect the whole body, including weakness, nervousness, fever, etc. (Table 5.1).

Phytochemical studies regarding *G. americana* — especially involving the isolation of their compounds — are still scarce. Brigas et al. (2016) pointed out the presence of some compounds as iridoids, phytosterols, and phenolics, all associated with different therapeutic properties. Among the iridoids isolated from mature fruits of genipap, the genipin deserves mentioning (Djerassi et al. 1960) due to its anti-inflammatory and antiangiogenic properties demonstrated by Ueda and Iwahashi (1991) and Koo et al. (2004).

Other compounds isolated from *G. americana*, including geniposide, tarenoside, gardenoside and geniposidic acid exhibited anticancer activity in tumor-induced from the Ragi cell line by Epstein-Bar virus (Ueda and Iwahashi 1991). In addition, genipin and geniposide demonstrated anti-inflammatory activity in carrageenan-induced duck edema in rats (Koo et al. 2004). Additional pharmacological activities of iridoids have been proved by scientific studies, such as hypotensive, spasmolytic, antiarrhythmic, hepatoprotective, hypoglycemic, hypolipidemic, antitumor, antiviral, immunomodulatory, among others.

Costa et al. (2010) reported that the seeds and the pulp of genipap fruit contain phytosterols such as campesterol, stigmasterol, and β-sitosterol. Phytosterols are present in genipap in high concentrations and have the ability to lower serum cholesterol levels of Low-Density Lipoprotein (LDL). Phenolic extracts obtained from *G. americana* fruits were evaluated by MTT [3(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium], MUH (4-methylumbelliferylheptanoate) and Methylene, indicating significant antiproliferative activity for HepG2 hepatocarcinoma (Finco et al. 2013).

A long-standing tradition in Brazil involves the mixing of various herbs in '*cachaça'* (sugar cane brandy), a mixture called "*garrafada*". Herbal sellers generally recommend the use of small daily doses, usually one small cup (30 mL) per day (Benko-Iseppon et al. 2012), with genipap fruits, bark or leaves being one of the elements frequently used in these infusions. Marques et al. (2015) reported the use of genipap in '*garrafadas*' together with six species (popular names *'catuaba', 'jurubeba', 'jurema-preta', 'pau-ferro', 'prá-tudo',* and '*ginseng'*). The indication of use included treatment of chronic diseases; osteoporosis; osteitis; osteomyelitis; periosteitis and back pain. Another survey by Nogueira (2005) reported the association of up to 20 herbs in a bottle sold in open markets in Rio de Janeiro, indicated against weakness (male or female) to "*cleanse the blood*", for problems of the digestive tract and for women who have difficulty in getting pregnant.

We have noted that ethnobotanical surveys rarely report on these mixtures or on the associations of various plant species in a single product, although they are highly demanded and consumed by the Brazilian population. Among the possible reasons for this lack of studies is the difficulty of identifying the contents of these bottles, especially of establishing the botanical species they contain (Benko-Iseppon et al. 2012, Marques et al. 2015). In addition, the possible cytotoxic and allelopathic and mutagenic effects

of these mixtures have been poorly studied. Marques et al. (2015) highlight the lack of standardization between different bottles, the poor information in the labels (if any available), emphasizing that the sale of these products do not comply with legal regulations. We observed a similar situation for syrups sold in the markets of the northeastern Brazilian region, with the lack of labels or in some cases including only the popular name of species and not following the labeling regulations required.

It is important to encourage herbal producers and sellers involved in the production chain of these products about the necessary availability of information and demand for standardization, also to preserve valuable ethnobotanical information for the next generations.

Adverse effects and toxicity analyses

The importance of a careful evaluation of the cytotoxic, mutagenic and genotoxic potential of medicinal plants and their compounds using *in vitro* and *in vivo* systems is evident, what can be achieved by different types of tests using diverse types of model organisms (Araújo et al. 2015). Despite this importance, only three evaluations were carried out to verify the possible adverse effects of *G. americana*. According to Assis (2015), the hydroethanolic extract of the fruits of *G. americana* showed no *in vivo* toxicity (acute and subchronic toxicity) for mice. Similarly, Omena et al. (2012) found that the pulp and seeds of genipap are rich in antioxidants and did not present cytotoxic activity to ovine corneal epithelial cells. However, Fernandez et al. (2011) reported that the extract prepared from leaves of genipap significantly inhibited proliferation in the meristematic cells of *Allium cepa*. In addition, the same extract also promoted inhibition of the cell cycle in fibroblasts of the cell line NCTC-929.

Considering the available analyses, it is possible to assume that there are significant differences between compounds from different genipap tissues, requiring additional studies, including analyses of diverse dosages and types of extracts. Particular care should be taken especially in the case of leaves that present apparent toxicity, although this tissue represents only the third most consumed plant part (as tea, infusion or maceration). It should be noted that no studies were found on the toxicity of the trunk/bark, although this part is indicated as the second most used for therapeutic applications (Table 5.1, Fig. 5.3a).

Genipap fruit as food and source of raw material

The only part of genipap considered edible for humans is the fruit. Due to their strong taste, it is usually said in Brazil that fresh fruits of genipap can arouse two types of reaction from consumers: love or hate, not appealing to most people. Therefore, the mature fruits of genipap are rarely consumed *in natura*. They are generally used as raw material for the production of jams, crystallized fruits, ice cream, soft drinks, in the manufacture of liquor, jellies, juices, wines, sweets and syrups (Silva and Tassara 2005, Andrade et al. 2016). Besides their characteristic flavor and aroma, these fruits are rich in iron, vitamins B1, B2, B5, and C, also containing calcium and carbohydrates in their composition. Genipap fruit is a major source of minerals as well as other bioactive compounds for the human diet (Vasco et al. 2008).

Fruit histochemical and chemical evaluations carried out by Figueiredo et al. (1986) reported that genipap pulp presents low acidity, high moisture content, low protein and lipid content, high sugar content, regular iron content, proper calcium and phosphorus content, high tannin content and palmitic and linoleic acids (fatty acids) as well as traces of vitamin C and pectin.

Analyzing the frozen pulp of genipap, Souza et al. (2012) found values of 4.46% of carbohydrates, 0.21% of proteins, 0.34% of lipids and 1.15% of dietary fibers. Also the presence of minerals such as Potassium (K) 92.55 mg/100 g, Calcium (Ca) 13.23 mg/100 g, Magnesium (Mg) 8.17 mg/100 g and Iron (Fe) 0.22 mg/100 g was reported, proving to be a major source of nutrients.

Genipap as a source of pigments

Most reports on the traditional use of genipap regard the use of immature fruits that deliver a blue dye employed in diverse types of application. This property of the fruits was already well known to Amerindians who used them to tattoo their bodies in religious ceremonies, during battles, to dye fabrics, ornaments,

ceramics, spears and other artifacts (Gade 2016). Studies have shown that when the unripe fruit is cut, and its interior is exposed to the air, its pulp gradually becomes dark, acquiring an intense blue color (Cavalcante 1991). In this way, Indians extract the juice of the genipap that at first has a citrus color, turning to green, violet, blue, dark blue and finally almost black. The blue pigment is formed from the reaction between genipin, a colorless iridoid present in genipap, and primary amine sources such as amino acids and proteins (Touyama et al. 1994, Bentes and Mercadante 2014).

According to Corrêa (1984), navigators and European colonizers who reached ports in different points of America are unanimous in registering the appreciation with which the juice was treated by the natives, from Brazil to Mexico and the Antilles. Tattoos last a few weeks, and there are paintings on artifacts and pictographs on rocks that have survived through the centuries to the present day.

Often the genipap dye was associated with the red dye '*urucum*' (*Bixa orellana* L., Bixaceae). There are reports that Catholic missionaries disapproved of such tattoos and fought against body paintings by requiring the natives to wash before entering the churches. In the case of '*urucum*' tattoos could be washed, but the genipap tincture only disappears with time, and for this reason, the prohibitions were often dismissed (Corrêa 1984). Cavalcante (1991) reported that according to J. Huber there are testimonies from South American natives stating that *G. americana* was more valued and cultivated by Amerindians because of its dye than as a source of food or wood.

Currently, there are some cosmetics for application to the hair and skin, that are available which are recommended as temporary tattoo based on the dye of genipap, with a similar appearance to the tattoos under the skin and estimated duration of 15 days.

Wood exploitation

The genipap wood has a light-colored core, extremely flexible, straight fibers and relative durability. It has an average density of 0.68 g/cm³, being considered moderately heavy, flexible, compact, easy to work and long lasting. It is not considered as hardwood but is much appreciated in woodworking, woodcutting, civil and naval construction, carpentry for the manufacture of furniture, firearm stock, tool handles, and door and window frames, being highly valued in curved pieces such as bows of barrels, tennis rackets, sieves, etc. (Corrêa 1984, Barbosa 2008, Santos 2012). Other authors reported the use of genipap's bark, which is rich in tannins and is used in tanneries in the treatment of leather (Erbano and Duarte 2010, Moura et al. 2016).

Despite its promising characteristics, there is no genipap cultivation for the timber industry. Almeida (2013) highlights the use of wood in construction and vessels in the northeastern region of Brazil in an irregular manner, without any kind of preservation measure. We highlight that in this region there is considerable fragmentation of the Atlantic forest, which was replaced by sugarcane fields, with great impact on tree species such as genipap.

This species has been also indicated as valuable in urban and pastureland afforestation, providing shade and shelter to the animals, being also used as forage (Salman et al. 2008, Santos et al. 2015), since its leaves are well accepted by animals, including cattle, pigs, and goats (Table 5.2). Other studies have demonstrated the ability of the genipap tree for phytoremediation, especially due to its capacity to absorb chromium (a heavy metal), harmful to living organisms (Barbosa et al. 2007, Santana et al. 2012). Thus, this plant can be a valuable tool in the regeneration of areas contaminated with this metal, persistent in the surroundings of leather tanneries, being considered carcinogenic in its hexavalent form.

Patents associated to *G. americana*

Aiming to identify existing patents from *G. americana* we carried out a search using the keyword "*Genipa americana*" in the US Patent and Trademark Office (USPTO) database, recovering 16 patents related to uses of this species. The patents were categorized according to their applications, including (1) Production of organic or synthetic compounds; (2) Method for production of organic compounds; (3) Method for production of compounds rich in genipin; (4) Compositions and methods for hair coloring; (5) Multiple emulsion for the stabilization of natural dyes and (6) Phytosanitary formulation for plant defense (Fig. 5.4).

Table 5.2 Non-medicinal uses of *G. americana* reported in the literature.

Sector	Plant part	Uses and applications	Source
Human nutrition	Mature fruits	Production of jam; crystallized fruit, soda, juice, frozen pulp, syrup, liquor, wine, alcohol, vinegar, brandy and ice cream, etc.	Corrêa 1984, Epstein 2001, Santos 2001, Prudente 2002
Wood industry	Wood	Shipbuilding; construction of buildings; luxury carpentry; in cooperage, in foundries (molding of parts), in woodcut, etc.	Corrêa 1984, Epstein 2001, Almeida 2013
Leather tanning	Wood bark	Extract used for bathing in leather pieces (tanning).	Epstein 2001, Erbano and Duarte 2010, Moura et al. 2016
Leather tanning	Immature fruit	Extract used for bathing in leather pieces (tanning).	Epstein 2001
Reforestation	Whole plant	Recovery of deforested areas, provision of shade, formation of living fences and street afforestation.	Epstein 2001
Animal feed	Leaves and mature fruits	Feeding of cattle, goats, and pigs.	Epstein 2001, Pinto-Ruiz et al. 2005, Salman et al. 2008
Phytoremediation	Whole plant	Phytostabilizer and chrome filterer, soil recovery.	Barbosa et al. 2007, Santana et al. 2012
Urban landscaping	Whole plant	Planting of seedlings in malls and squares.	Pinto-Ruiz et al. 2005, Salman et al. 2008, Santos et al. 2015

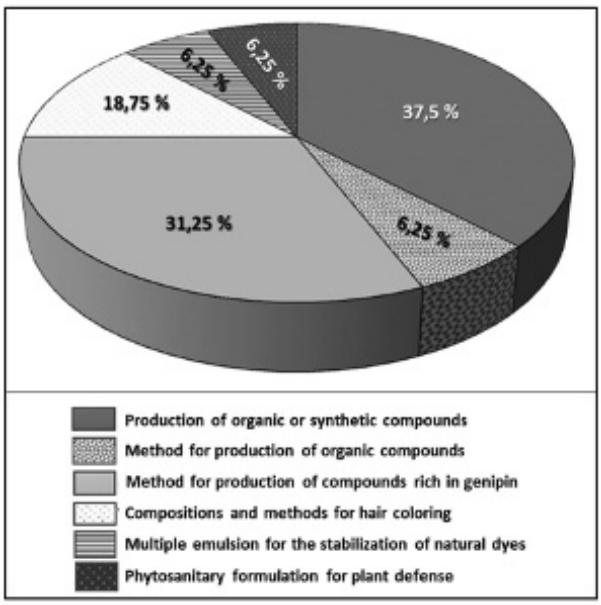


Fig. 5.4 Categories of patents deposited in the US Patent and Trademark Office (USPTO) databank involving *Genipa americana* (http://www.uspto.gov/; access in June 2017).

The first patents related to *G. americana* were deposited between the 1980s and 1990s and mostly referred to methods of isolation or production of new organic or synthetic compounds obtained from plant extracts for pharmaceutical or pharmacological application. Such patents report the potential of iridoid derivatives (monoterpene compounds that occur in plants), such as antihyperlipidemic and cholagogue, and point to their potential use in the formulation of cholesterol-lowering drugs (Table 5.3, Fig. 5.4).

Table 5.3 Patents related to *Genipa americana* available in the database US Patent and Trademark Office (USPTO).

Patent #	Title	Description	Inventor	Deposited in
RE46,314	Genipin-rich material and its use	Method for producing new genipin-rich compounds obtained from *G. americana* fruit for application in the development of dyes.	Chr. Hansen Natural Colors A/S	February 2017
9,427,007	Multiple emulsions for colorants	Water-oil-water multiple emulsion for the stabilization of natural colorants of food, pharmaceutical, and cosmetic products.	Chr. Hansen Natural Colors A/S	August 2016
9,376,569	Colorant derived from genipin	Isolation method and characterization of dye compounds obtained from genipin or genipin and amine for use as a dye in foods, medicines, cosmetics, medical equipment and textile products.	Ecoflora S.A.S.	June 2016
8,980,793	Phytosanitary formulations	A phytosanitary formulation and method comprising an active ingredient, an alcoholic, hydroalcoholic or aqueous extract produced naturally by dyeing plants with applications in agriculture and horticulture as herbicide, insecticide or fungicide.	Univ. Blaise Pascal-Clermont-Ferrand II, CNRS	March 2015
8,945,640	Genipin-rich material and its use	Method for producing new genipin-rich compounds obtained from *G. americana* fruit for application as a cross-linking reagent and as a raw material in the development of colorants.	WILD Flavors, Inc.	February 2015
8,632,612	Compositions for dyeing keratin fibers	Compositions for coloring keratin fibers and their manufacturing methods for application in the cosmetics industry.	Segetis, Inc.	January 2014
8,557,319	Stable natural color process, products and use thereof	Method of producing dyes from the processing of *G. americana* juice with other edible juices or extracts containing nitrogen compounds for application in the beverage, food coloring, pharmaceutical industry, dietary supplements, cosmetics, personal hygiene and animal food industry.	WILD Flavors, Inc.	October 2013
7,927,637	Blue colorant derived from *G. americana* fruit	Method of production of liquid or powdered blue dye obtained from *G. americana* unprocessed natural juice for application in the textile, pharmaceutical, food, cosmetic and other industries.	Ecoflora SA	April 2011
7,699,897	Method of hair coloring	Compositions and methods for hair coloring for application in the cosmetics industry.	L'Oreal	April 2010
5,459,160	Iridoid derivatives and the use thereof as a drug	Use of derivatives of iridoids synthesized from genipin with antihyperlipidemic action and collagen for pharmaceutical application.	Tsumura & Co.	October 1995
5,374,653	Iridoid derivatives and the use thereof as a drug	Use of derivatives of iridoids synthesized from genipin with antihyperlipidemic action and collagen for pharmaceutical application.	Tsumura & Co.	December 1994

Table 5.3 contd....

...Table 5.3 contd.

Patent #	Title	Description	Inventor	Deposited in
5,272,172	Iridoid derivatives and the use thereof as a drug	Use of derivatives of iridoids synthesized from genipin with antihyperlipidemic action and collagen for pharmaceutical application.	Tsumura & Co.	December 1993
4,347,356	Novel nitrogen-containing monoterpene derivatives	Use of monoterpene derivatives with nitrogen-containing a pyridine chain, dimers, trimers and higher polymers as efficient and low toxicity dyes for pharmacological applications.	Taito Co., Ltd.	August 1982
4,247,698	Red coloring composite and the method for its production	Method and production of a reddish-colored compound obtained from the reaction of iridoid compounds and biological substances for application as a food pigment.	Taito Co., Ltd.	January 1981
4,232,159	Nitrogen-containing monoterpene derivatives	Use of monoterpene derivatives with nitrogen-containing a pyridine chain, dimers, trimers and higher polymers as low toxicity dyes for pharmacological applications.	Taito Co., Ltd.	November 1980
3,932,628	Extracts from active tree saps	Production of new organic compounds obtained from dried extracts of sap from trees with antileukemic action in mice.	US Department of Health	January 1976

With advances in research, the use of these compounds was expanded to other areas such as food, beverages, pharmaceuticals, dietary supplements, cosmetics, textiles, personal care products and animal feed. This was the second most representative group of the search, with five patents for the methods and production of compounds rich in genipin for application as dyes in the mentioned areas, emphasizing the significant phytochemical potential of genipap (Table 5.3, Fig. 5.4).

The third group, with two patents deposited in 2010 concerned hair coloring compositions and methods. This group emphasizes the pursuit of the cosmetic industry by plant raw materials capable of providing low-cost, low-toxicity natural dyes as an alternative to minimize the irritating or toxic effects of synthetic substances present in hair dyes, also allowing the creation of new shades for use in this market.

Other deposited patents included the production of a reddish coloring compound for use as a dye or pigment in foods, a multiple emulsion for the stabilization of natural dyes, and a phytosanitary formulation applied in agriculture and horticulture (Table 5.3, Fig. 5.4). The formulation of natural defensive substances for phytosanitary use stands out as a new application directed to the agronomic area, presenting the herbicide, insecticide or fungicide potential, which could minimize the toxic effects caused by commercial insecticides for the health of workers and consumers of agricultural products, as well as for the environment.

It is interesting to note that many of the proposed uses and products patented are in consonance with the indications and uses made by native communities in Latin America, emphasizing the great importance of ethnobotanical studies in the indication of potential targets for bioprospecting of new compounds and products of plant origin. Also worth mentioning is the tendency towards a greater appreciation of natural products over synthetic products, in the area of cosmetics, food colorants, fabric dyeing, and in defense against pests and diseases of agriculture, adding value to natural products, including derivatives of genipap. This paradigm shift is made clear by the filing of nine recent patents for 'non-toxic' products of natural origin between 2010 and 2017. The last patent before this new tendency was in 1995 (Table 5.3). Such a shift is possibly associated with the clear consumer demands for natural products and ecologically sound sources.

Conservation, cultivation and extractive practices

The wide geographic distribution and good adaptability of *G. americana* in several tropical regions (Lorenzi and Matos 2008) reinforces its ability to grow under diverse environmental conditions. In addition, this species has been shown to be promising in the recovery of deforested and degraded areas due to its adaptive characteristics to the ciliary environment, its rapid growth, abundant seed production and its capacity to withstand long periods in wet soils, becoming a target of forestry studies (Barbosa et al. 1989, Sebbenn 1997, Rabbani et al. 2012).

According to Ferreira et al. (2005), due to its multiplicity of uses, and high potential for immediate use among native fruit plants, genipap was included among the 10 species selected as priority by the program 'Plants of the Future' a joint initiative of the Brazilian Ministry of the Environment in conjunction with the Global Environment Facility of the World Bank.

Although genipap is a species of recognized productive potential and socioeconomic relevance, works evaluating its cultivation and productivity are still very limited. In addition, extractive practices have been the main way of obtaining plant material, making the genipap tree very vulnerable and susceptible to forest fragmentations and genetic erosion, which has contributed to a reduction of its diversity in some areas. For example, Santos (2012) reported that the current practice is to preserve the trees that produce good fruit, and those that do not serve this purpose are transformed into wood for various uses in the clandestine timber market. Thus, the extractive activities are related to the broad range of applications of this species and its logging characteristics (Santos et al. 2011, Bessa et al. 2013).

Currently, extractive practices have become one of the main threats to plants with edible fruits, being carried out in a predatory way by practically complete removal of quality fruits (Ribeiro and Walter 2008). Moreover, this fact accentuates the risk of extinction of these genotypes, making it essential to target conservation and domestication efforts for species of environmental and economic interest (Gomes and Moura 2010).

Aiming at the conservation of diversity, strategies for multiplication, conservation, and sustainable management are essential since these species constitute an important source of genetic resources (Paunescu 2009). In this sense, studies evaluating germination capacity and seed storage are mandatory (Carvalho

and Nascimento 2000, Prado Neto et al. 2007, Magistrali et al. 2013), besides *in vitro* cultivation and regeneration trials, as well as acclimatization of seedlings (Rocha et al. 2008, Yee et al. 2010, Almeida 2013, Almeida et al. 2015).

A major initiative involved the creation in 2009 of an Active Germplasm Bank of Genipap Tree by Embrapa (Brazilian Agricultural Research Company) Coastal Tablelands station in Sergipe state, Northeastern region of Brazil. This station includes an experimental field, where plants of different origins are kept, aiming at their preservation (Almeida 2013). The maintenance of genetic resources of *G. americana* is of particular importance considering its problematic physiology, since the seeds tolerate desiccation only at low levels (Carvalho and Nascimento 2000, Oliveira et al. 2011), presenting low viability and absence of germination after 60 days of storage (Vieira and Gusmão 2006). Despite this initiative, efforts to preserve the species are still limited, requiring higher investments in research and development.

Conclusions and future perspectives

As highlighted throughout this chapter, since the earliest times of human civilization a communion exists between genipap and the human populations in the regions where this species occurs. All parts of the plant are used, with emphasis on its fruits (mature or immature) and the trunks. There are several products (some patented) that, interestingly, reflect the primary uses of the parts of this plant. Considering the Convention on Biological Diversity (CBD) and the Nagoya Protocol, the knowledge and role of traditional populations in relation to their genetic resources should be recognized. The CBD proclaims three main objectives: (1) conservation of biological diversity; (2) the sustainable use of its constituent parts; (3) the fair and equitable sharing of benefits arising from the utilization of genetic resources (Rabitz 2015).

However, the scenario for achieving these goals is not promising in the case of genipap, since many of their areas of occurrence have been devastated and replaced by plantations and pastures, with emphasis on the Atlantic and Amazon forests, as well as Cerrado's riparian forests in Brazil.

With regard to sustainability, there are still no established and implemented methodologies for the propagation and scale plantation of *G. americana*. In this way, its use has been associated with extractive practices or the maintenance of some individuals in pastures and backyards of private properties. Appraisement of some populations in the last 15 years by our group draws attention to the lack of succession by young plants in the surroundings of existing trees older than 40 years or more. In addition, the fragments where the trees occur are gradually decreasing in size, plant density and diversity. Besides, as reported, few seeds germinate, and most of the seedlings die few days or weeks after germination. Therefore, it is imperative to analyze the reproductive mechanisms of this species, which may help in the definition of a strategy for the production of useful seedlings or stocks for reforestation and to maintain their diversity.

In regard to "the fair and equitable sharing of benefits arising from the use of genetic resources", the reality is still far from achieving this goal. Practically all patents listed in this chapter derived from information associated with popular knowledge of genipap, show the importance of ethnobotanical surveys in different regions of its occurrence, especially those with greater dependence on natural resources. If the benefits are to be shared, it remains to be seen by the next generations.

References

Agra, M.F., Freitas, P.F. and Barbosa-Filho, J.M. 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Braz. J. Pharmacog. 17: 114–140.

Almeida, C.S. 2013. Propagação e conservação *in vitro* de acessos de jenipapeiro. M.Sc. Dissertation, Universidade Federal de Sergipe, Brazil. Available at https: //bdtd.ufs.br/bitstream/tede/656/1/camila_santos_almeida.pdf.

Almeida, C.S., Silva, A.V.C., Araújo, A.G. and Lédo, A.S. 2015. Respostas morfogenéticas de jenipapeiro em diferentes condições de cultura *in vitro*. Rev Caatinga 28: 58–64.

Alves, E.O., Mota, J.H., Soares, T.S., Vieira, M.C. and Silva, C.B. 2008. Levantamento etnobotânico e caracterização de plantas medicinais em fragmentos florestais de Dourados–MS. Ciênc. Agrotec. 32: 651–658.

Andrade, A.C.S., Souza, A.F., Ramos, F.N., Pereira, T.S. and Cruz, A.P.M. 2000. Germinação de sementes de jenipapo: temperatura, substrato e morfologia do desenvolvimento pós-seminal. Pesq. Agropec. Bras. 35: 609–615.

Andrade, S.A.C., Metri, J.C., Barros Neto, B. and Guerra, N.B. 2016. Osmotic dehydration of jenipapo (*Genipa americana* L.). Food Sci. Technol. 23: 276–281.

Andreasen, K. and Bremer, B. 1996. Phylogeny of the subfamily Ixoroideae (Rubiaceae). Opera Bot. Belg. 7: 119–138.

Andreasen, K. and Bremer, B. 1997. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: morphology and chloroplast DNA data. *In*: Andreasen, K. (ed.). Phylogeny of Ixoroideae: Molecular Systematics within Rubiaceae. Ph.D. Dissertation, Uppsala University, Uppsala, Sweden.

Araújo, S.S., Fernandes, T.C.C., Marin-Morales, M.A., Brasileiro-Vidal, A.C. and Benko-Iseppon, A.M. 2015. Mutagenicity, genotoxicity and cytotoxicity assays of medicinal plants: first step for drug development. pp. 130–153. *In*: Duarte, M.C.T. and Rai, M. (eds.). Therapeutic Medicinal Plants: From lab to the market. CRC Press, New Hampshire, United Kingdom.

Assis, C.S. 2015. Avaliação dos efeitos tóxicos *in vitro* e *in vivo* do extrato hidroetanólico dos frutos de *Genipa americana* L. (Rubiaceae) em camundongos Swiss. M.Sc. Dissertation, Universidade Federal do Rio Grande do Norte, Brazil. 73 pp. Available at https: //repositorio.ufrn.br/jspui/bitstream/123456789/20796/1/cinthyasaraivadeassis_dissert.pdf.

Barbosa, D.A. 2008. Avaliação fitoquímica e farmacológica de *Genipa americana* L. (Rubiaceae). M.Sc. Dissertation, Universidade Federal do Rio de Janeiro, Brazil. 136 pp. Available at http: //objdig.ufrj.br/59/teses/726901.pdf.

Barbosa, L.M., Batista, E.A., Mantovani, W., Veronese, S.A. and Andreani, R. 1989. Ensaios para estabelecimento de modelos para recuperação de áreas degradadas de matas ciliares, Mogi-Guaçú (SP). pp. 268–283. Nota Prévia. *In*: Simpósio sobre Mata Ciliar, 1. Campinas. Fundação CARGILL.

Barbosa, R.M.T., Almeida, A.A.F., Mielke, M.S., Loguercio, L.L., Mangabeira, P.A.O. and Gomes, F.P. 2007. A physiological analysis of *Genipa americana* L.: a potential phytoremediator tree for chromium polluted watersheds. Environ. Exp. Bot. 61(3): 264–271.

Barroso, G.M., Guimarães, E.F., Ichaso, C.L.F., Costa, C.G., Peixoto, A.L. and Lima, H.C. 1991. Rubiaceae. pp. 189–229. *In*: Sistemática de Angiospermas do Brasil. Universidade Federal de Viçosa, Viçosa, Brazil.

Benko-Iseppon, A.M. and Crovella, S. 2010. Ethnobotanical bioprospection of candidates for potential antimicrobial drugs from Brazilian plants: state of art and perspectives. Curr. Protein Pept. Sci. 11(3): 189–194.

Benko-Iseppon, A.M., Pinangé, D.S.B., Chang, S.C. and Morawetz, W. 2012. Ethnobotanical uses of the native flora from Brazilian North-Eastern Region. pp. 84–105. *In*: Rai, M., Rastrelli, L., Marinoff, M., Martinez, J.L. and Cordell, G. (eds.). 2012. Medicinal Plants: Biodiversity and Drugs. 1st. ed. CRC Press, New Hampshire.

Bentes, A.D.S. and Mercadante, A.Z. 2014. Influence of the stage of ripeness on the composition of iridoids and phenolic compounds in genipap (*Genipa americana* L.). J. Agric. Food Chem. 62: 10800–10808.

Bessa, N.G.F., Borges, J.C.M., Beserra, F.P., Carvalho, R.H.A., Pereira, M.A.B., Fagundes, R., Campos, S.L., Ribeiro, L.U., Quirino, M.S., Chagas Junior, A.F. and Alves, A. 2013. Prospecção fitoquímica preliminar de plantas nativas do cerrado de uso popular medicinal pela comunidade rural do assentamento Vale Verde – Tocantins. Rev. Bras. Pl. Med. 15: 692–707.

Borges, E. and Rezende, C.M. 2000. Main aroma constituents of genipap (*Genipa americana* L.) and bacuri (*Platonia insignis* M.). J. Essent. Oil Res. 12(1): 71–74.

Braga, R. 1960. Plantas do Nordeste (especialmente, do Ceará). 2 ed. Imprensa Oficial, Fortaleza, p. 540.

Bremer, B. 2009. A review of molecular phylogenetic studies of Rubiaceae. Ann. Missouri Bot. Gard. 96: 4–26.

Bremer, B. and Eriksson, T. 2009. Time tree of Rubiaceae: Phylogeny and dating the family, subfamilies, and tribes. Int. J. Pl. Sci. 170: 766–793.

Brigas, S., Costa, E. and Cabrita, S. 2016. The influence of *Genipa americana* L. on the adipose tissue of mice. Exp. Pathol. Health Sci. 8: 19–22.

Camargo, N.B., Gontijo, O.J.S. and Barbosa, A.C.O.F. 2016. Characterization of microsatellite regions in native plants of the Brazilian. Multi-Sci. J. 2(6): 41–47.

Candolle, A.P de. 1830. Prodromus systematis naturalis regni vegetabilis. Vol. 4. Treuttel & Würz, Paris.

Carvalho, J.E.U. and Nascimento, W.M.O. 2000. Sensibilidade de sementes de jenipapo (*Genipa americana* L.) ao dessecamento e ao congelamento. Rev. Bras. Frutic. 22: 53–56.

Cavalcante, P.B. 1991. Frutas Comestíveis da Amazônia**.** Coleção Adolfo Ducke, 5th ed. CEJUP/Museu Paraense Emílio Goeldi, Belém, p. 279.

Codignoto, P.S.C., Araújo, S.B., Bastos, N.M., Fernandes, T.O., Barbosa, T.A.S., Igidio, C.E.D., Faustino, F., Fernandes, M.J.B. and Conceição. A.O. 2017. *In vitro* cytotoxicity and biological activities of *Genipa americana* (Rubiaceae) ethanolic extracts. Afr. J. Microbiol. Res. 11: 385–390.

Conceição, A.O., Rossi, M.H., Oliveira, F.F., Takser, L. and Lafond, J. 2011. *Genipa americana* (Rubiaceae) fruit extract affects mitogen-activated protein kinase cell pathways in human trophoblast–derived BeWo Cells: Implications for placental development. J. Med. Food 14: 483–494.

Cordeiro, J.M.P. and Félix, L.P. 2014. Conhecimento botânico medicinal sobre espécies vegetais nativas da caatinga e plantas espontâneas no agreste da Paraíba, Brasil. Rev. Bras. Plantas Med. 16: 685–692.

Corrêa, P.M. 1984. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. IBDF, Rio de Janeiro, p.747.

Corrêa, A.M. and Forni-Martins, E.R. 2004. Chromosome studies of species of Rubiaceae (A.L. de Jussieu) from the Brazilian cerrado. Caryologia 57: 250–258.

Costa, P.A., Ballus, C.A., Teixeira-Filho, J. and Godoy, H.T. 2010. Phytosterols and tocopherols content of pulps and nuts of Brazilian fruits. Food. Res. Int. 43: 1603–1606.

Delprete, P.G., Smith, L.B. and Klein, R.M. 2005. Flora Ilustrada Catarinense: Rubiáceas. Herbário Barbosa Rodrigues, Itajaí.

Dias, S.R.K., Mendonça, A.C.A.M. and Silva, M.A.P. 2013. Ethnobotanical, phytochemical and pharmacological aspects Rubiaceae species in Brazil. RCPM 18: 140–156.

Djerassi, C., Gray, J.D. and Kincl, F. 1960. Isolation and characterization of genipin. J. Org. Chem. 25: 2174–2177.

Djerassi, C., Nakano, T., James, A.N., Zakow, L.H., Eisenbraun, E.J. and Shoolery, J.N. 1961. Terpenoids. XLVII. The structure of Genipin. J. Org. Chem. 26: 1192–1206.

Donadio, L.C., Nachtigal, J.C. and Sacramento, C.K. 1998. Frutas exóticas. FUNEP, Jaboticabal, p. 279.
Epstein, L. 2001. Cultivo e aproveitamento do jenipapo. Rev. Bah. Agr. 4: 23–24.
Erbano, M. and Duarte, M.R. 2010. Morfoanatomia de folha e caule de *Genipa americana* L., Rubiaceae. Braz. J. Pharmacog. 20: 825–832.
Fernandez, V., Franco de Diana, D., Fernández, D., Vega Gómez, M.C., Segovia Abreu, J., Castiglioni, D., Sales, L., Martinez, M., López, D., Bobadilla, N., Alfonso, J., Mojoli le quesne, A., Monges, D. and Vera, M. 2011. Efecto antimitótico y citotóxico del extracto etanólico de *Genipa americana* L. sobre tejido meristematico de *Allium cepa* L. y cultivo celular NCTC-929. Steviana 3: 48–53.
Ferreira, E.G., Lemos, E.E.P., Souza, F.X., Lourenço, I.P., Lederman, I.E., Bezerra, J.E.F., Silva-Junior, J.F., Barros, L.M., Rufino, M.S.M. and Oliveira, M.E.B. 2005. Frutíferas. pp. 49–100. *In*: Sampaio, E.V.S.B., Pareyn, F.G.C., de Figueirôa, J.M. and Santos Junior, A.G. (eds.). Espécies da flora nordestina de importância econômica potencial. Associação Plantas do Nordeste. Recife. Brazil.
Figueiredo, R.W., Maia, G.A., Holanda, L.F.F. and Monteiro, J.C.S. 1986. Características físicas e químicas do jenipapo. Pesq. Agropec. Bras. 21: 421–428.
Finco, F.D.B.A., Boser, S. and Graeve, L. 2013. Antiproliferative activity of Bacaba (*Oenocarpus bacaba*) and Jenipapo (*Genipa americana* L.) phenolic extracts a comparison of assays. Food Sci. Nutr. 43: 98–106.
Francis, J.K. 1993. *Genipa americana* L. Jagua, genipa. U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, New Orlands, LA, p.5.
Gade, D. 2016. Spell of the Urubamba: Anthropogeographical Essays on an Andean Valley in Space and Time. Springer International Publishing, Switzerland, p.354.
Gentry, A.H. 1993. A field guide to the families and genera of woody plants of Northwest South America (Colombia, Ecuador, Peru) with supplementary notes on herbaceous taxa. Conservation International, Washington, DC, p.895.
Gibbs, P.E. and R. Ingram. 1982. Chromosome numbers of some Brazilian flowering plants. Notes Roy. Bot. Gard. Edinburg 40: 229–407.
Gomes, C.C. and Moura, T.M. 2010. Estrutura genética em populações de plantas do Cerrado. Agrotec. 1: 33–51.
Guerra, M. 1986. Citogenética de Angiospermas coletadas em Pernambuco. Braz. J. Genet. 9: 21–40.
Guerra, M. 1993. Patterns of heterochromatin in a tropical tree species: *Genipa americana* L. (Rubiaceae). Cytologia 58: 427–432.
Guerra, M. 2001. Patterns of heterochromatin distribution in plant chromosomes. Genet. Mol. Biol. 23: 1029–1041.
Kainulainen, K., Razafimandimbison, S.G. and Bremer, B. 2013. Phylogenetic relationships and new tribal delimitations in subfamily Ixoroideae (Rubiaceae). Bot. J. Linn. Soc. 173: 387–406.
Kiehn, M. 1986. Karyologische Untersuchungen und DNA-Messungen an Rubiaceae und ihre Bedeutung fur die Systematik dieser Familie. Dissertation zur Erlangung des Doktorgrades, Universität Wien.
Koo, H.J., Song, Y.S., Kim, H.J., Lee, Y.H., Hong, S.M., Kim, S.J., Kim, B.C., Jim, C., Lim, C.J. and Park, E.H. 2004. Antiinflammatory effects of genipin, an active principle of gardenia. Eur. J. Pharmacol. 495: 201–208.
Lorenzi, H. 2002. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. Instituto Plantarum, Nova Odessa, São Paulo, p.368.
Lorenzi, H. and Matos, F.J.A. 2002. Plantas medicinais no Brasil: nativas e exóticas. Instituto Plantarum, São Paulo, p.512.
Lorenzi, H. and Matos, F.J.A. 2008. Plantas medicinais no Brasil: Nativas e Exóticas. Instituto Plantarum, Nova Odessa, São Paulo, p.544.
Magistrali, P.R., Faria, J.A.C. and Gasparin, E. 2013. Physiological behavior of *Genipa americana* L. seeds regarding the capacity for desiccation and storage tolerance. J. Seed Sci. 35(4): 495–500.
Manoel, R.O. 2014. Fluxo gênico, estrutura genética espacial intrapopulacional e sistema de reprodução hierárquico entre e dentro de frutos em uma pequena população isolada de *Genipa americana* L., utilizando marcadores microssatélites. Ph.D. Thesis, Universidade Estadual Paulista Júlio de Mesquita Filho, São Paulo, Brazil. 96 pp. Available at https://repositorio.unesp.br/bitstream/handle/11449/123312/000826564.pdf?sequence=1&isAllowed=y.
Manoel, R.O., Freitas, M.L.M., Barreto, M.A., Moraes, M.L., Souza, A.P. and Sebbenn, A.M. 2014. Development and characterization of 32 microsatellite loci in *Genipa americana* (Rubiaceae). Appl. Plant Sci. I 2(3): 1–6.
Manoel, R.O., Freitas, M.L.M., Tambarussi, E.V., Cambuim, J., Moraes, M.L.T. and Sebbenn, A.M. 2015. Mendelian inheritance, genetic linkage, and genotypic 57 disequilibrium at microsatellite *loci* in *Genipa americana* L. (Rubiaceae). Gen. Mol. Res. 14: 8161–8169.
Marques, J.O., Oliveira, M.F.F. and Lacerda, G.A. 2015. Efeito alelopático e análise dos rótulos de garrafadas comercializadas no Mercado Municipal de Montes Claros. Rev. Bras. Plant. Med. 17(4): 1134–1141.
Matos, F.J.A. 1999. Plantas da medicina popular do nordeste. UFC, Fortaleza, p.80.
Moreira, R.C.T., Costa, L.C.B., Costa, R.C.S. and Rocha, E.A. 2002. Ethnobotanical approach on the use of medicinal plants in Vila Cachoeira, Ilhéus, Bahia, Brasil. Acta Farm. Bonaer. 21: 205–211.
Mors, W.B., Rizzini, C.T. and Pereira, N.A. 2000. Medicinal Plants of Brazil. Reference Publications, Inc. Algonac, Michigan, p.501.
Mouly, A., Kainulainen, K., Persson, C., Davis, A.P., Wong, K.M., Razafimandimbison, S.G. and Bremer, B. 2014. Phylogenetic structure and clade circumscriptions in the Gardenieae complex (Rubiaceae). Taxon. 63(4): 801–818.
Moura, R.S. de. 2014. Diversidade genética e germinação de jenipapo. M.Sc. Dissertation, Universidade Federal de Sergipe, Sergipe, Brazil. 67pp. Available at https: //bdtd.ufs.br/bitstream/tede/542/1/rafaela_santos_moura.pdf.
Moura, S.M.S., Sousa, S.R.S. and Conde Júnior, A.M. 2016. *Genipa americana* L.: prospecção tecnológica. JIBI 1: 31–35.

Nascimento, W.M.O., Carvalho, J.E.U. and Carvalho, M.M. 2000. Germinação de sementes de jenipapo (*Genipa americana*), submetidas a diferentes temperaturas e substratos. Ver. Bras. Frut. 22: 471–473.

Nogueira, A.J. 2005. Medicina Popular. Prefeitura Municipal, Rio de Janeiro, p.49.

Nogueira, F.A., Nery, P.S., Morais-Costa, F., Oliveira, N.J.F., Martins, E.R. and Duarte, E.R. 2014. Efficacy of aqueous extracts of *Genipa americana* L. (Rubiaceae) in inhibiting larval development and eclosion of gastrointestinal nematodes of sheep. J. Appl. Anim. Res. 42: 356–360.

Oliveira, L.M., Silva, E.O., Bruno, R.L.A. and Alves, E.U. 2011. Períodos e ambientes de secagem na qualidade de sementes de *Genipa americana* L. Semina (Ciênc. Agrar.). 32(2): 495–502.

Omena, C.M.B., Valentim, I.B., Guedes, G.S., Rabelo, L.A., Mano, C.M., Bechara, J.H., Sawaya, A.C.H.F., Trevisan, M.T.S., Costa, J.G., Ferreira, R.C.S., Sant'ana, A.E.G. and Goulart, M.O.F. 2012. Antioxidant, anti-acetylcholinesterase and cytotoxic activities of ethanol extracts of peel, pulp and seeds of exotic Brazilian fruits Antioxidant, anti-acetylcholinesterase and cytotoxic activities in fruits. Food Res. Int. 49: 334–344.

Paunescu, A. 2009. Biotechnology for endangered plant conservation: a critical overview. Rom. Biotechnol. Lett. 14: 4095–4103.

Persson, C. 1996. Phylogeny of the Gardenieae (Rubiaceae). Bot. J. Linn. Soc. 121: 91–109.

Persson, C. 2000a. Phylogeny of Gardenieae (Rubiaceae) based on chloroplast DNA sequences from the rps16 intron and trnL(UAA)-F(GAA) intergenic spacer. Nord. J. Bot. 20(3): 257–269.

Persson, C. 2000b. Phylogeny of the neotropical Alibertia group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. Amer. J. Bot. 87: 1018–1028.

Persson, C. 2003. *Agouticarpa*, a New Neotropical Genus of Tribe Gardenieae (Rubiaceae). Brittonia. 55(2): 176–201.

Pierozzi, N.I. and Da Cruz, N.D. 1988. Número e identificação dos cromossomos de *Genipa americana* L. (Rubiaceae) através da técnica de banda-C. Ciên. Cult. 40: 802.

Pierozzi, N.I. and Mendaçolli, S.L.J. 1997. Karyotype and C-analysis in two species of *Genipa* L. (Rubiaceae, Gardenieae Tribe). Cytologia 62: 81–90.

Pinto-Ruiz, R., Gómez, H., Martínez, B., Hernández, A., Medina, F.J., Gutiérrez, R., Escobar, E. and Vázquez, J. 2005. Árboles y arbustos forrajeros del sur de Mexico. Pastos y Forrajes. 28(2): 87–97.

Prado Neto, M., Dantas, A.C.V.L., Vieira, E.L. and Almeida, V.O. 2007. Germinação de sementes de jenipapeiro submetidas à pré-embebição em regulador e estimulante vegetal. Ciênc. Agrotec. 31(3): 693–698.

Prudente, R.M. 2002. Jenipapo (*Genipa americana* L.). pp. 88–114. *In*: Vieira Neto, R.D. (ed.). Fruteiras Potenciais Para os Tabuleiros Costeiros e Baixadas Litorâneas, Embrapa-CPATC/ENDAGRO, Aracajú, Brazil.

Rabbani, A.R.C., Silva-Mann, R. and R.A. Ferreira. 2012. Genetic variability of *Genipa americana* L. belonging to the lower course of São Francisco river. Rev. Arv. 36: 401–409.

Rabitz, F. 2015. Biopiracy after the Nagoya Protocol: problem structure, regime design and implementation challenges. Bras. Polit. Sci. Rev. 9(2): 30–53.

Ribeiro, J.F. and Walter, B.M.T. 2008. Fitofisionomias do bioma Cerrado. pp. 151–212. *In*: Sano, S.M., Almeida, S.P. and Ribeiro, J.F. (eds.). Cerrado: Ecologia e Flora, Embrapa-CPAC, Planaltina, Brazil.

Robbrecht, E. and C. Puff. 1986. A survey of the Gardenieae and related tribes (Rubiaceae). Bot. Jahrb. Syst. 108: 63–137.

Robineau, L. 1995. Hacia Una Pharmacopea Caribeña. TRAMIL 7 endacaribe UAG & Universidade de Antioquia Santo Domingo, Santo Domingo, p.696.

Rocha, M.A.C., Costa, M.A.P.C., Silva, S.A., Lédo, C.A.S., Moreira, M.J. and Bastos, L.P. 2008. Enraizamento *in vitro* e aclimatização de genótipos de jenipapeiro (*Genipa americana* L.). Rev. Bras. Frutic. 30(3): 769–774.

Salman, A.K.D., López, G.F.Z., Bentes-Gama, M.M. and Andrade, C.M.S. 2008. Espécies arbóreas nativas da Amazônia Ocidental Brasileira com potencial para arborização de pastagens. Embrapa Rondônia, Porto Velho, Documentos, Vol. 127, p.23.

Salomão, A.N. and Allem, A. 2001. Polyembryony in angiospermous trees of the Brazilian Cerrado and Caatinga vegetation. Acta Bot. Bras. 15: 369–378.

Santana, K.B., Almeida, A.A.F., Souza, V.L., Mangabeira, P.A.O., Silva, D.C., Gomes, F.P., Dutruch, L. and Loguercio, L.L. 2012. Physiological analyses of *Genipa americana* L. reveals a tree with ability as phytostabilizer and rhizofilterer of chromium ions for phytoremediation of polluted watersheds. Environ. Exp. Bot. 80: 35–42.

Santos, R.O.S. 2001. Caracterização de jenipapeiros (*Genipa americana* L.) em Cruz das Almas – BA. M.Sc. Dissertation, Universidade Federal da Bahia, Brazil, 65pp.

Santos, P.A. 2012. Manejo do jenipapeiro (*Genipa americana* L.) para produção de madeira e avaliação da diversidade genética por meio de marcadores moleculares, Cruz das Almas-Bahia. M.Sc. Dissertation, Universidade Federal do Recôncavo Baiano, Bahia, Brazil. 40pp. Available at https://www1.ufrb.edu.br/pgrecvegetais/images/...pedro_de_almeida.pdf.

Santos, A.R.F., Silva-Mann, R.S. and Ferreira, R.A. 2011. Restrição hídrica em sementes de jenipapo (*Genipa americana* L.). Rev. Arv. 35: 213–220.

Santos, C.Z.A., Ferreira, R.A., Santos, L.R., Santos, L.I., Gomes, S.H. and Graça, D.A.S. 2015. Análise qualitativa da arborização urbana de 25 vias públicas da cidade de Aracaju – SE. Ci. Fl. 25(3): 751–763.

Sebbenn, A.M. 1997. Estrutura genética de subpopulações de *Genipa americana* L. (Rubiaceae) a partir de Isoenzimas. Doctoral Thesis, Universidade de São Paulo, Brazil, 107pp. Available at http://repository.usp.br/single.php?_id=001031068.

Sebbenn, A.M., Kageyama, P.Y. and Vencovsky, R. 1998. Estrutura genética de subpopulações de *G. americana* L. (Rubiaceae) em mata ciliar a partir de isoenzimas. Ver. Inst. Flor 10: 95–108.

Sebbenn, A.M., Kageyama, P.Y. and Vencovsky, R. 2003. *In situ* genetic conservation and number of tree for seed collect in *Genipa americana* L. population. Sci. For. 63: 13–22.

Sebbenn, A.M. 2004. Herança e ligação em locos de isoenzimas em *Genipa americana* L. Rev. Inst. Flor 16: 65–72.
Silva, S. and Tassara, H. 2005. Frutas Brasil frutas, 4th ed. Empresa das Artes, São Paulo, p.321.
Silva, A.P., Lima, C.L.C. and Vieites, R.L. 1998. Caracterização química e física do jenipapo (*Genipa americana* L.) armazenado. Piracicaba. Sci. Agric. 55: 29–34.
Silva, A.V.C., Freire, K.C.S., Lédo, A.S. and Rabbani, A.R.C. 2014. Diversity and genetic structure of jenipapo (*Genipa americana* L.) Brazilian accessions. Sci. Agric. 71(5): 345–355.
Silva, A.V.C., Muniz, E.N., Almeida, C.S., Vitória, M.F., Ledo, A.S., Melo, M.F.V. and Rabbani, A.R.C. 2015. Genetic diversity and sex identification in *Genipa americana* L. Trop. Subtrop. Agroecosyt. 18: 81–86.
Souza, V.R., Pereira, P.A.P., Queiroz, F., Borges, S.V. and Carneiro, J.D.S. 2012. Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits. Food Chem. 134: 381–386.
Telles, M.P.C. and Bastos, R.P. 2009. Estruturação genética na escala de paisagem. Megadiversidade 5(1-2): 101–110.
Touyama, R., Takeda, Y., Inoue, K., Kawamura, I., Yatsuzuka, M., Ikumoto, T., Shingu, T., Yokoi, T. and Inouye, H. 1994. Studies on the blue pigments produced from genipin and methylamine. I. Structures of the brownish-red pigments, intermediates leading to the blue pigments. Chem. Pharm. Bull. 42: 668–673.
Ueda, S. and Iwahashi, Y. 1991. Production of anti-tumor-promoting iridoid glucosides in *Genipa americana* and its cell cultures. J. Nat. Prod. 54: 1677–1680.
Vasco, C., Ruales, J. and Kamal-Eldin, A. 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chem. 111: 816–823.
Vieira, F.A. and Gusmão, E. 2006. Efeitos de giberelinas, fungicidas e do armazenamento na germinação de sementes de *Genipa americana* L. (Rubiaceae). Cerne. 12(2): 137–144.
Xavier, M. 1976. Jenipapo: uma espécie indígena para reflorestar. Cerrado 8: 20–23.
Yee, T.F., Goh, C.J. and Rao, A.N. 2010. *In vitro* studies on *Genipa americana*. JTMP 11: 71–88.
Zappi, D.C., Semir, J. and Pierozzi, N.I. 1995. *Genipa infundibuliformis* sp. Nov. and notes on *Genipa americana* (Rubiaceae). Kew Bull. 50: 761–771.

6

Paramela (*Adesmia boronioides* Hook. f.)

From Popular Uses to Commercialization

Silvia B. González,[1,*] *Ana H. Ladio,*[2] *Bruno Gastaldi,*[1] *Fresia M. Silva Sofrás,*[1] *Ariel Mazzoni*[3] and *Gustavo Sánchez*[4]

Introduction

Adesmia boronioides Hook.f. is a perennial bush belonging to the Leguminosae family (Fabaceae). Its common name is "paramela" and is a species with a long history of use among the native societies of the Argentinean-Chilean Patagonia. Due to its cultural and symbolic value, it stands out as part of the biocultural heritage of the region. It is part of the knowledge and practices related mainly with the health and subsistence of Mapuche and Tehuelche communities since pre-hispanic times (Molares and Ladio 2009, Ciampagna and Caparelli 2012). It is a plant used for human consumption as medicine (Martínez-Crovetto 1980, Campos et al. 1997, Montes et al. 2001) as well as ornamental and melliferous (Forcone and Muñoz 2009, Green and Ferreyra 2011).

Recently, commercial interest towards this plant has increased given its exceptional conditions and potential, and in particular due to its fragrant odor (since 2005, its essential oil has been used as a supply for the perfume industry). Nowadays, paramela is used in rural areas mainly as a medicinal infusion, and in urban areas as ornament and/or as an aromatic ingredient for the preparation of an alcoholic beverage.

This native plant of the Patagonian region inhabits low irrigation sites, is of slow growth, and its culture is of interest (Contardi et al. 2016a, 2016b). So far it is almost exclusively found in its natural state (Barthelemy et al. 2008). However, in a great part of the rural communities, mainly those of Mapuche-Tehuelche ascendance, the paramela is protected in family orchards either because it is tolerated and cared for with the rest of the plants or because it has been transplanted to these spaces, thus being possible to place it in an incipient domestication process (Ladio and Molares 2017).

A successful and sustained development of commercial products derived from native plants requires a domestication process of the species. This process would ensure homogenous genetic material and high

[1] Universidad Nacional de la Patagonia San Juan Bosco sede Esquel, Facultad de Ciencias Naturales y Ciencias de la Salud, Ruta Nacional N°259 km 16,41 (U9200) Esquel, Chubut, Argentina.
[2] Grupo de Etnobiología. Instituto de Investigaciones en Biodiversidad y Medio Ambiente CONICET.
[3] Estación Experimental Agropecuaria Bariloche, Instituto Nacional de Tecnología Agropecuaria (INTA).
[4] Universidad Nacional de Río Negro, Sede Andina, Tecnicatura en Viveros.
* Corresponding author: quim-esq@unpata.edu.ar

quality specimens. It would involve an improvement in the raw materials and a standardization of the content in the active principles. Reproduction studies made from *A. boronioides* seeds, allowed for the development of the species propagation protocols and the production of plants in greenhouses (González et al. 2009, Sánchez and Riat 2012, Mazzoni et al. 2014). Since 2015, an experimental culture in the Andean region of the Argentinean Patagonia allows for the evaluation of the productivity and quality of the cultivated plant's essential oil in relation to the wild population.

It is of particular interest to point out the negative consequences of unreasonable commercial exploitation of the wild populations of the species and the substantial importance of their cultivation. In the latter case, further studies are needed regarding the selection of chemotypes and their population dynamics, as well as major research on the possible management and sustainable use of wild populations. This is essential in order to be able to establish manuals of good practices for those who are engaged in their collection.

Botanical and ecological information of the species

Botanical description

A. boronioides is a perennial bush belonging to the Leguminosae family (Fabaceae). Its common name is "paramela". Its habitat ranges from 0 m.a.s.l. to 2,200 m.a.s.l. It has a medium size, varying between 0.40–2 m. It is highly ramified, with glandular branches, fragrant and very resinous and sticky to the touch. It has an axonomorph root. The leaves are 3–6 cm, shortly petiolate, 10–20-leaflets, leaf rachis with erect, brief hair; obovate, fleshy, glabrous, toothed, shiny, 4–6 mm leaflets with crateriform glands especially at the edge; short, amplexicaul, glabrous, glandular stipules. Clusters of 4–7 cm, densiflorous, sessile, ovate, acute, glandular, glabrous bracts. The flowers are 7–10 mm, colorful, yellow, perfumed, with campanulate chalice, pubescent, glandulous, with short teeth, serice-pubescent in their interior; glabrous vexillum (banner), glabrous wings and keel shorter than vexil. Ovary with some marginal hair. Narrow, pubescent, glandular, 3–5-articulate loments; semicircular, dehiscent 4.5–6 mm trusses.

Its anatomy was initially studied by Nájera et al. (2000). Subsequently, in an evaluation of leaves and stems of diverse origins in their Patagonian distribution, very similar microscopic characters were found. They all have cyclocytic stomata on the leaves' epidermis. The secretory pores are located, in greater number, on the abaxial surface, although some pores can be observed on the adaxial surface. On some samples, secretion pores were found at the end of the leaves semilimbos (e.g., Los Antiguos, El Calafate, Bariloche, Villa La Angostura) (González et al. 2014).

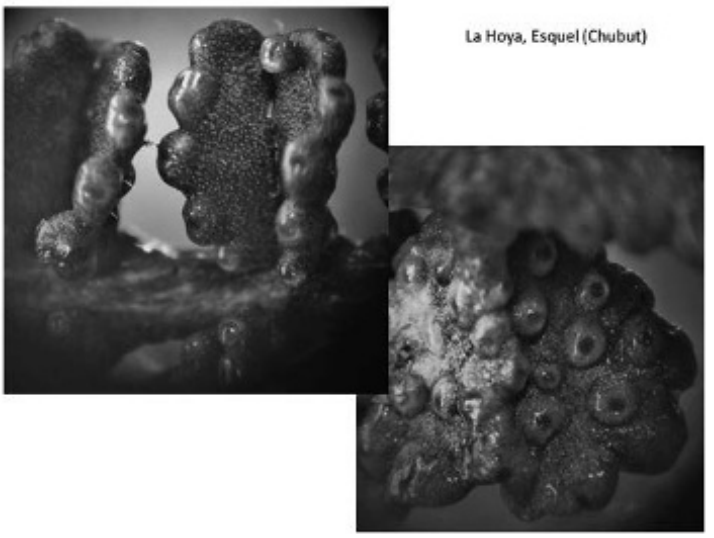


Fig. 6.1 Detail of the glands in the bundle and the back of the leaflets (10x). Photograph by González, S.B.

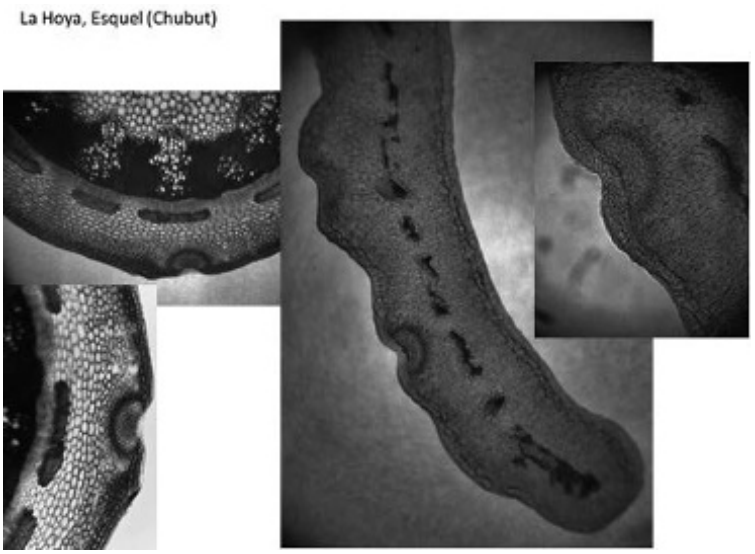


Fig. 6.2 Microscopic structure of the glandular structures in stems and leaves (40x) Guerra P.E. Photograph by González, S.B.

Geographic distribution

A. boronioides's distribution ranges from Mendoza to Tierra del Fuego provinces, in Argentina, including also Neuquén, Rio Negro, Chubut, and Santa Cruz, and the XI and XII Regions of Chile (http://www2.darwin.edu.ar/Proyectos/FloraArgentina/fa.htm, Burkart 1967, Ulibarri and Burkart 2000).

It inhabits sunny areas, shrubs, river sides, roads and ravines, mainly in the Patagonian steppe, shrubland areas and steppe-forest transition zones (https://www.sib.gov.ar/ficha/PLANTAE*adesmia*boronioides). The forest areas where paramela can be found consist mainly of *lenga* (*Nothofagus pumilio*), ñire (*Nothofagus antarctica*) and cypress (*Austrocedrus chilensis*) (Molares and Ladio 2012b). It has also been found in the Atlantic littoral, in Santa Cruz province, in the Rio Gallegos area (González et al. 2014). In slopes, near Los Molles, Mendoza, it grows in shrubland vegetation.

Generalities of the *Adesmia* genus

The *Adesmia* DC genus has only been found in South America, in approximately 240 species. These species are distributed mainly in the center of Chile and in the south and west regions of Argentina. In this country, more than 100 representative species have been found, which makes it the most numerous genus of the Leguminosae, Papilionoideae. There are about 55 species in the Patagonian region (Burkart 1984, Ulibarri and Burkart 2000, Ulibarri and Simpson 2010).

A. boronioides stands out as the only species of this highly glandulous-resinous genus (Burkart 1967).

Ethnobotany

Its common name is paramela, but it is also known as *té silvestre* (wild tea), *yerba carmelita* (Carmelite herb), *éter* (ether), *pegapega, yagneu* and *lonkotrevo* (González 2002, Molares and Ladio 2012a,b). Native people of Patagonia have great respect for this plant, and use it for both medicinal and symbolic purposes. Its use is inscribed within a holistic understanding of health in which different participants, elements and unique worldviews that have no equivalent in western science are involved (Ladio and Molares 2014, 2017). For this reason, a "translation" of certain uses from a western perspective must be done carefully since they could be oversimplified or interpreted as therapeutically inaccurate.

This plant is used as medicine by a large part of the Patagonian rural population. Its medicinal use has been registered in ethnobotanical works by communities very distant from each other (Neuquén, Rio

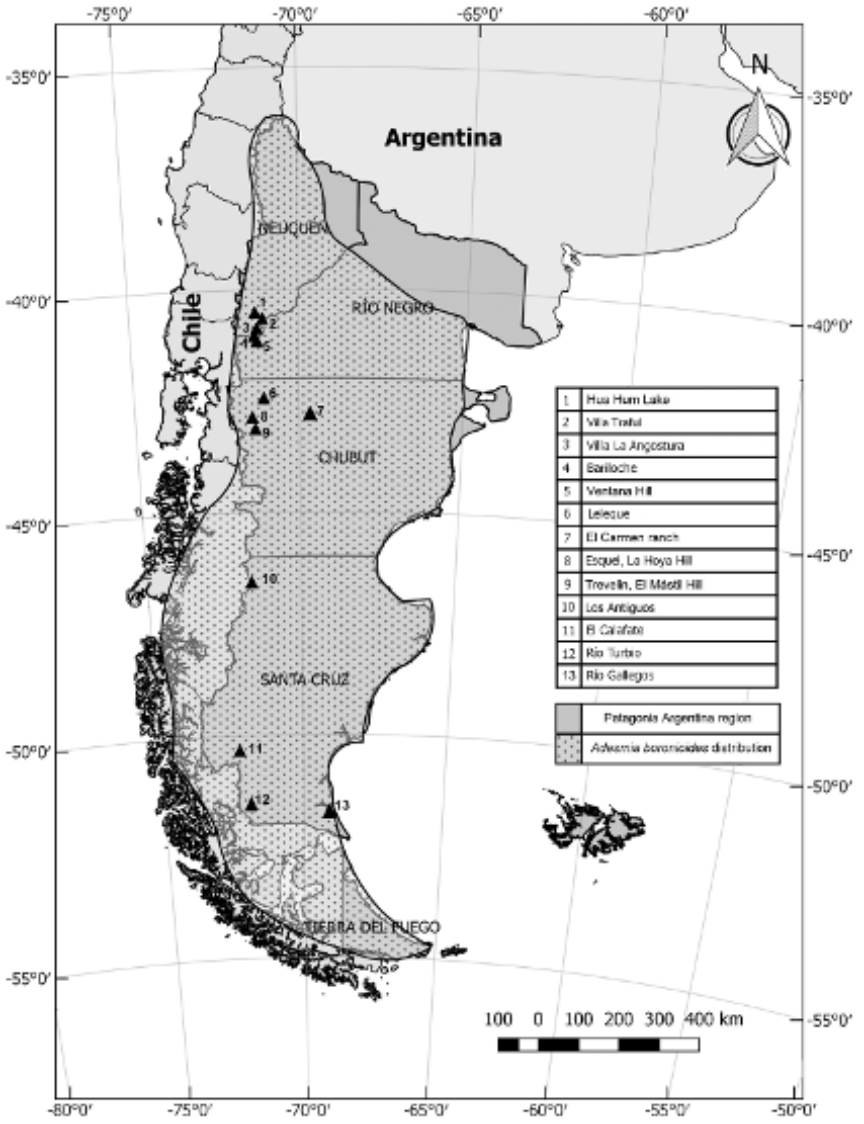


Fig. 6.3 *A. boronioides* distribution map. Neri, A. and Gastaldi, B.

Negro and Chubut provinces, as well as the south regions of Chile) (González et al. 2005, Molares and Ladio 2009). In most studies conducted in Mapuche-Tehuelche communities, the species has high levels of consensus among inhabitants, in many cases greater than 75%, that is to say, their knowledge and use of the plant is widely shared and disseminated among the rural population (Ladio 2006, Molares and Ladio 2014).

The species has been most recurrently cited as digestive, antirheumatic, diaphoretic and antiemetic (Campos et al. 1997, González et al. 2004, 2005, Toledo and Kutschker 2012, Molares and Ladio 2014). Today, it is a substantial part of these communities' home medicine, being part of the first-aid kit of rural families that use it to cope with health problems autonomously (Richeri et al. 2013). Traditionally, it is collected in autumn-winter, while the animals are taken care of, and kept dry in paper bags in dark places to use it as medicine during the whole year (Richeri et al. 2013). The people of the countryside distinguish it by its "perfumed" character and it is listed in the group of plants with "magical soul" and "sweet smell and bitter taste" (Molares and Ladio 2009, Ladio and Molares 2014).

It has been used since ancient times by the native people of the region mainly to relieve bruises, sprains, cramps, and joint and muscle pains (González 2002, Estomba et al. 2006, Igon et al. 2006). It is also valued for having the property of "heating the body" when prepared in the form of steam inhalations and baths, with the plant submerged in hot water (González 2005). These baths are mainly used to prevent children from urinating in bed when it is cold (Igon et al. 2006). Also, the steam inhalations are used against cold and cough discomfort, in communities of Neuquén, Río Negro and Chubut provinces (Igon et al. 2006, Eyssartier et al. 2011, Richeri et al. 2013). According to Richeri (2016), families in the Chubut plateau gather paramela to treat asthmatic people or for bronchitis cases. This author also records the use of the plant as incense (burning), perfume and to cleanse the houses of evil spirits. According to Ochoa (2005), the species is also used in ointment as a sedative for rheumatic problems and to heal wounds.

The tea or infusion of paramela is widely used as digestive (Igon et al. 2006). It is also included in the *mate*, a traditional drink of the region that is mainly composed by *mate* herbs (*Ilex paraguariensis*) (Weigandt et al. 2004). It is marketed in businesses dedicated to the sale of medicinal plants in S.C. de Bariloche mainly for its digestive properties (Cuassolo et al. 2010). The infusion is also used to wash the hair in order to kill lice and is considered to be a hair vitalizer (Martínez-Crovetto 1980, Conticello et al. 1997, Igon et al. 2006). Ochoa (2008) describes that, in Arroyo Las Minas (Rio Negro), the infusion is used for kidney problems, and women drink the infusion after childbirth to recover faster, as an invigorating drink. The use for kidney problems has also been cited by Kutschker et al. (2002). Some studies have also identified it as an aphrodisiac (Muñoz et al. 2001, Igon et al. 2006). In Comallo and Pilcaniyeu (Rio Negro), people emphasize the use of the plant's decoction to ease flu, stomach pains, diarrhea, and fever (Eyssartier et al. 2009, 2011, 2013).

Popular uses of paramela

Infusion (tea): In most local recipes it is prepared using a tablespoon of leaves per cup. The dose is one cup per day.

Mate: Fresh/dry leaves are added to the mate infusion.

Decoction: The twigs are boiled in water and eaten with burnt sugar or alone.

Plaster: The leaves are crushed and embedded in a gauze or rag that is placed over the sore area.

Baths and steam inhalations: A large bowl is filled with hot water and branches of the plant are added.

Incense: Branches are placed on the kitchen stove or burned slowly to release its fragrance.

Other local uses

It is cited as an excellent forage (Green and Ferreyra 2011). In their work on the Chubut plateau, Castillo and Ladio (2014) found that it is a plant used as forage for goats and sheep, even though it is considered by the shepherds as a problematic plant, because the animal's meat will have an unpleasant taste after feeding on this plant. Also, Green and Ferreyra (2011) described its moth-repellent properties.

Chemical constituents

The first chemical analysis of essential oils and resinoids obtained from leaves and stems was made in 1963 (Montes and Peltz 1963). They described physicochemical properties of the essential oils and chromatograms, but without mentioning any specific compounds due to the instrumental limitations of the time.

Agnese et al. (1989, 1993) made phytochemical studies on some *Adesmia* species (*A. grandiflora, A. bicolor, A. retrofracta, A. trijuga, A. horrida, A. incana, A. aegiceras*). They analyzed hydrophilic compounds including flavonoids, pinitol, vanillin and glucose, and some lipophilic compounds such as alkanes, carboxylic acids and wax.

Faini et al. (1995) reported three new triterpene glycosides of the malabaricane type in *A. aconcaguensis*.

The main component structure in the essential oil is a bisnorsesquiterpene called "esquelenone". This component was named after the city "Esquel", in the Argentinean Patagonia, where paramela grows naturally. Taking into account the stereochemistry of the compound, the name is (1S,4R,5S)-esquel-6-en-9-one, whereas the IUPAC name is (3aS,1R,7aS)-1,2,3,3a,5,7a-hexahydro-1-methyl-6-(1-methylethyl)-4H-inden-4-one (Fig. 6.4).

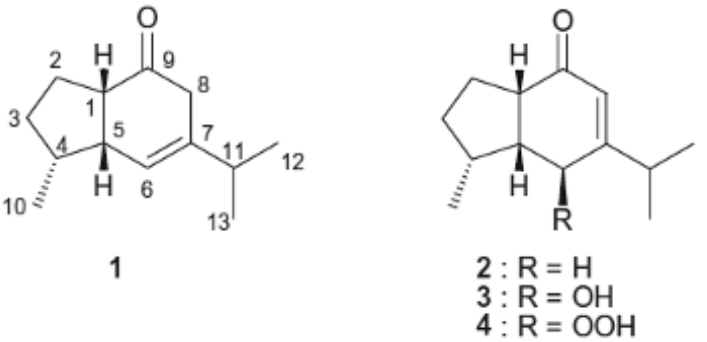


Fig. 6.4 Absolute configurations of esquelane derivatives 1–4 (Carlos M. Cerda-García-Rojas et al. 2015).

Essential oil composition of *A. boronioides*

The essential oil yielded 0.5% in average, through hydrodistillation of aerial parts. This oil is yellow-greenish color with a pleasant sweet odor, specific gravity 0.9690 and refraction index 1.4972 at 20°C, [α]= +6 (0.02; hexane). The essential oil had a high content of sesquiterpenoids. The main compounds had a novel bisnorsesquiterpene structure (González et al. 2002) and were named Esquelenone and Isoesquelenone. The other important compounds had cadinane and eudesmane skeletons, the first one belonging to α-copaen-11-ol and the other to 10-epi-γ-eudesmol.

The relative amounts of the main components were directly influenced by the recollection sites, phenological stage, drying time, season, among others.

The main compound might derive biogenetically from MW 236, an intermediary compound, precursor of furopelargones, proposed by Lukas (1964). In turn, this hypothetical structure might derive from a guaiane skeleton (6,9-guaiadiene, Fig. 6.5).

The olfactory profile of this essential oil was uncommon and interesting, with a sweet, woody and spiced odor, with strong fixation properties (González et al. 2002).

Structural elucidation of the main components were performed through NMR (mono and bidimensional), Vibrational Circular Dichroism (VCD) and its absolute configuration was recently reassigned (González et al. 2002, Cerda-García-Rojas et al. 2015).

Essential oil composition includes not only novel structural components (Fig. 6.6) but also new terpene skeletons not reported previously in other species (González et al. 2004).

The essential oil, some extracts, and the main component all have a nice, fruity odor suitable for perfumery production. A dermal sensitivity test showed it was harmless to the skin (González 2002). Also, some assays showed high stability of the essential oil at room temperature, even with solar exposition. These results endorse the potential use of this natural product in the production of fragrances. An unfavorable organoleptic modification was observed, however, when the essential oil was subjected to heat or exposed to air. Under these conditions, the esquelenone went through an isomerization process in the first case, and through a peroxidation process in the second case. This resulted in a decrease in the fresh and fruity odor.

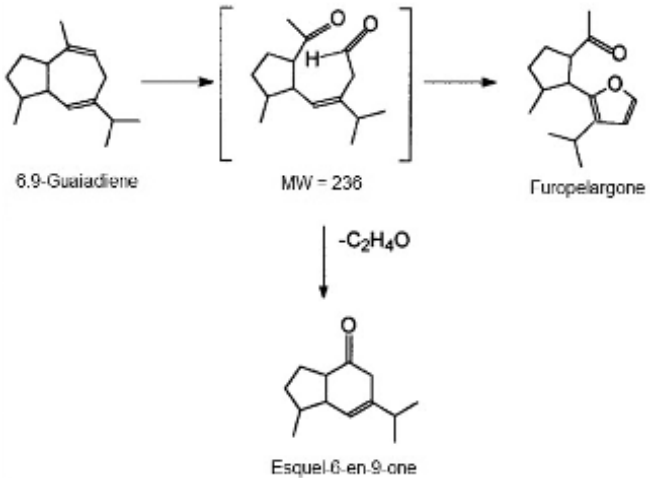


Fig. 6.5 Biosynthesis of Esquelenone (González 2002).

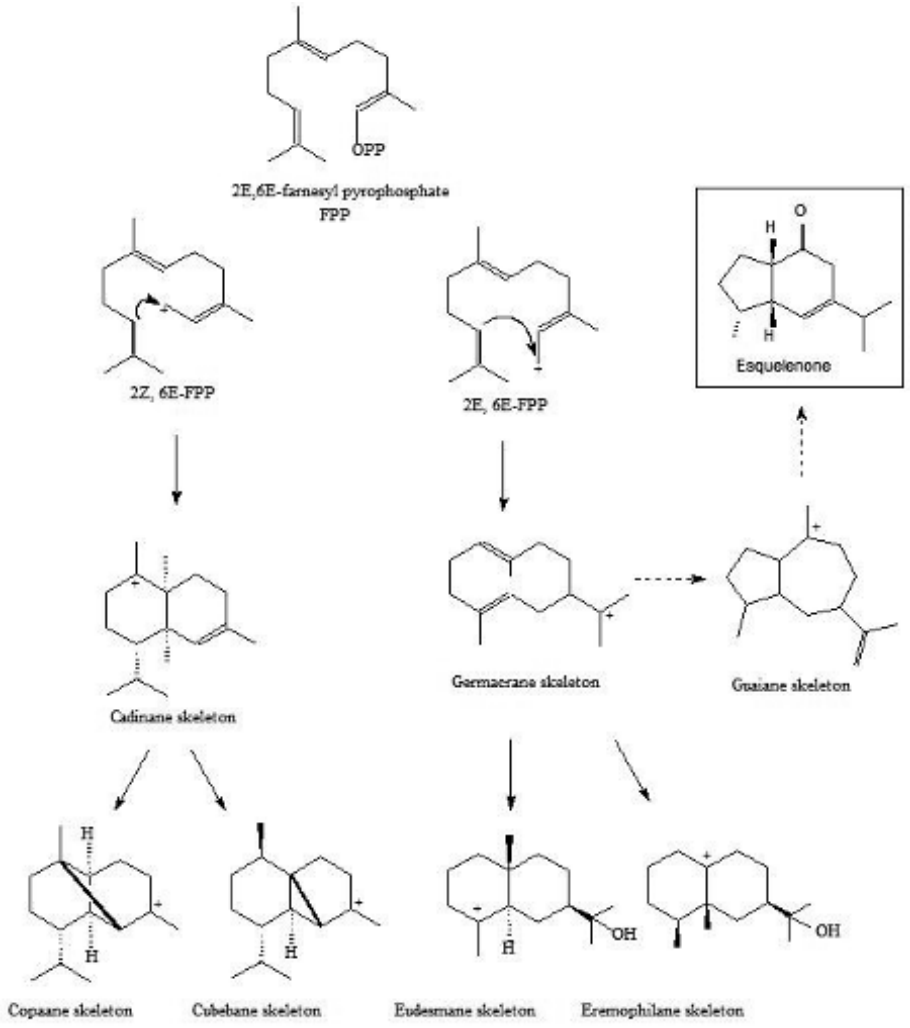


Fig. 6.6 Main sesquiterpenes skeletons in *A. boronioides's* essential oil (González 2002).

The esquelenone compound shares structural features with products which are being used currently in the perfumery industry such as 6,7-dihydro-1,1,2,3,3-pentamethyl 4-(5H)-indanone (Cashmeran®).

Chemical variability in the essential oil of *A. boronioides*

A study of the volatile compounds' chemical variability along the Patagonian distribution was conducted, including Neuquén, Río Negro, Chubut and Santa Cruz provinces in Argentina.

Contents and regulation of secondary metabolites are very sensitive to environmental influences and the presence of pathogens and predators. The chemical variability in natural populations of paramela in Patagonia has been confirmed. For example, it is remarkable to find a greater concentration of hemiterpenes (C_5) in the southernmost sites and the quantitative difference of cadinane skeletons, which are preponderant in some sites (La Hoya, Chubut 41% in 2015) and almost absent in others (Los Antiguos, Santa Cruz 0 to 3% in 2013–2015). Esquelanes/guaianes were quantitatively important in all studies, with a percentage of the total essential oil contents ranging from 27.4 to 60.4%. (González et al. 2016). Low molecular weight compounds were detected in Santa Cruz, Los Antiguos and El Calafate. The most noteworthy of these compounds was 2-methylbutanenitrile, precursor of cyanogenic glycosides, present in numerous species of plants and insects (González et al. 2014).

These findings show the importance of establishing the origin of the plant material in order to assure the essential oil quality.

Cyanogenic glycosides

The southernmost populations of *A. boronioides* analyzed (Los Antiguos y el Calafate) were the only ones which showed a positive reaction to Guignard test to detect cyanogenic glycosides. This is consistent with the presence of 2-methylbutanenitrile, lotaustralin precursor, in volatile compounds of *A. boronioides* in these locations (González et al. 2014).

According to the reference bibliography, cyanogenic plant populations were associated with lower altitude locations, which would point towards a negative correlation between altitude and cyanogenic plant number for a given species (de Araújo 1976, Richards and Fletcher 2002). This previous work was consistent with our findings, where the populations in altitudes lower than 300 m a.s.l. showed a positive reaction in the Guignard test (Silva Sofrás et al. 2016).

Paramela might be a polymorphic species regarding cyanogenic glycosides production (Kakes 1990) and its function would be related to different endogenous or exogenous factors, for example the presence of seed-feeding insects, very common in studied populations (Delfino et al. 2009).

The quantitative assay gave a maximum value of 0.47 µg HCN/g per plant. The lethal dose for an adult human is 30–120 mg HCN, so it would be necessary to ingest 65 kg of this plant to reach this level. *A. boronioides* is traditionally used in infusions, with barely 5 g in 100 ml of hot water, and therefore it is safe for human consumption.

Phenolic compounds content

There is only one previous study regarding paramela phenolic compounds (Silva Sofrás et al. 2016). In this work, 12 populations from different altitudes and latitudes in Patagonia were collected and analyzed in two seasons: spring and autumn. The antioxidant variability found by Gastaldi et al. (2016) is consistent with the total phenolic contents in each location.

The sites with altitudes lower than 500 m.a.s.l. had a higher value of total phenolic compounds in autumn and the ones above 500 m a.s.l. had a higher value of flavonoids in spring.

Total phenolic contents and antioxidant activity in *A. boronioides* are higher in autumn than in spring. There was a positive correlation between the number of compounds found and the site's latitude. Seventeen constituents were detected through qualitative analysis: three phenolic acids and 14 flavonoids.

The largest amount of compounds was detected in Rio Turbio (Santa Cruz), including a phenolic acid exclusive of this site. Three flavonoids were detected in Bariloche and Villa La Angostura which are exclusive of these sites.

There was no difference in the number of compounds detected in autumn and spring for the sites analyzed in both seasons.

The phenols and flavonoids qualitative analysis was consistent with total flavonoid content quantitative analysis (Silva Sofrás et al. 2016).

In vitro biological activity studies

Antioxidant activity

The aerial parts of the plant exhibited antioxidant activity, most likely due to the presence of phenolic compounds and flavonoids (Gastaldi et al. 2016, Silva Sofrás et al. 2016). Estomba et al. (2010) studied the antioxidant activity and pigments of *A. boronioides* using micropropagated 60-day seedlings from sterile cultured seeds. A low amount of total chlorophyll was observed with "a" chlorophyll reduction at the expense of the "b" chlorophyll (a/b chlorophyll: 2.98). Catalase activity (EC1.11.1.6) was low. The authors concluded that it is possible to use these *in vitro* cultures as a source of bioactive metabolites.

Trypanocidal activity

The concentration of *A. boronioides* that inhibited the growth of parasites by 50% was lower than the benzimidazole IC_{50}. In the inhibition curve, the trend line coincided with a polynomial equation, which would indicate that the growth of epimastigotes was affected by more than one variable. Villagra et al. (2008) concluded that new tests are required to support this hypothesis and suggested that it is possible to encourage the search for active compounds against Chagas disease from these essential oils.

Antimicrobial and antifungal activities

In vitro susceptibility assays and solid medium diffusion assays were carried out. For these assays, extracts in methanol, ethyl acetate, dichloromethane, hexane and water were prepared. The extracts were challenged with microorganisms (CCMA-29: Collection of Microbial Cultures of Argentina No. 29), impregnating filter paper disks of 5.5 mm up to a total load of 0.25 mg/mL. The culture media were at two pH (Antibiotic Medium I pH 6.6 and II and pH 7.9). The antimicrobial and antifungal activity under assay conditions was null, except for the extract in ethyl acetate, in medium at pH 6.6, which showed activity on *Staphylococcus aureus* (González 2002). In more recent studies (Blengini et al. 2016), antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 11198 was detected, with MIC values of 62.5 µg/mL for both bacteria. These studies were conducted with an experimental technique adapted from those suggested by the Clinical Laboratory Standards Institute (CLSI). A panel of positive and negative Gram bacteria was used and the oil was assayed. For the antifungal evaluation a broth microdilution method in front of a panel of standardized

fungi was used. The oil showed antifungal activity against *Candida glabrata* and *Candida parapsilosis*, with 1,000 µg/mL MIC values against both yeasts.

Anti-inflammatory activity

A methanolic extract, an infusion and the essential oil obtained by hydro distillation were assayed in accordance with the Argentine National Pharmacopoeia VII Edition.

The polar fractions (methanolic and aqueous) and the essential oil of *A. boronioides* were assayed at concentrations of 15 and 50 µg/mL for their eicosanoid generation effect (TBX2, PGE2 and LTB4) in rat peritoneal leukocytes. The methanolic extract and the essential oil showed a strong inhibition of LTB4 generation, whereas the aqueous extract was comparatively inactive. The methanolic extract showed potent TXB2 inhibition while the essential oil and aqueous extract were much less active. The effect on PGE2 production was smaller, implying that the greatest effect was produced on thromboxane synthase. The essential oil showed a significant LDH release in rat peritoneal leukocytes which would suggest a substantial toxicity to the cells; the other two extracts were not harmful.

Its anti-inflammatory activity has been tested *in vitro*, which would support one of the properties attributed to the plant, that is, as a "medicine" for rheumatic pains (González et al. 2003).

Acute toxicity studies

The potential acute toxicity of *A. boronioides* infusion was recently studied using the *Artemia salina* model. A 5% infusion was prepared from aerial parts of the plant following the standards of the Argentine National Pharmacopoeia VII Edition to make an infusion. A freeze-dried product was obtained from the infusion. The *A. saline* model organism was exposed to different concentrations of the freeze-dried product in order to obtain concentration-response curves and to determine lethal concentrations 50 (LC_{50}) in mg/mL.

$LC_{50} \leq 1$ mg/mL in the toxicity bioassay with *A. salina* is considered an acute toxicity indicator for an aqueous vegetal extract. This result can then be extrapolated to animals and humans. The value obtained from the *A. boronioides* assay was 5.16 mg/mL, which would indicate that an infusion made from this species would not present a risk of acute toxicity to humans (Mongelli et al. 1995, Pérez and Lazo 2010, Gastaldi et al. 2016).

Allergenicity: dermal irritability test

Tests performed with *A. boronioides* essential oil included the analysis of erythema (non-pruritic rash, bright red and slightly raised skin) and edema (accumulation of interstitial fluid in large amounts) in albino rabbit skin. The results showed the safety of the essential oil under the test conditions, according to the methodology of Draize (Gonzalez 2002).

Background of the species conservation state

In Argentina, the state of conservation of the paramela has not been systematically evaluated. According to IUCN its status is "not evaluated" (NE). However, in Chile it is in the red book of CONAF (Corporación Nacional Forestal, www.conaf.cl/) as a vulnerable species. In Argentina, there are no reliable studies so far, except for the fact that it is protected in all Patagonian National Parks (https://www.sib.gov.ar/ficha/PLANTAE*adesmia*boronioides). There is also no official information on the volumes that are subject to commercial exploitation to date, so we consider that their conservation status should be addressed and studied in more detail.

According to national and international standards (National Biodiversity Strategy and Plan of Action 2015–2020, IUCN and CBD), the use of goods should not affect the functioning and sustainability of ecosystems (flora, fauna, water, etc.), nor the cultural values of the local people. Consequently, if this species is to be used for commercial purposes, it is necessary to provide mechanisms that will ensure that this natural resource is used in the context of sustainable development, taking into account the rights

and values of local communities. This is the current perspective on conservation, where the protection of biodiversity is intimately related to socio-cultural and economic components through the concept of sustainable development.

Additionally, it is important to consider the protection of the different wild populations of this species, given that, due to its geographical extent, it shows morphological and chemical variations, particularly in its essential oil (González et al. 2016).

Commercial use

Today, the species is commercialized in three ways: (1) as an ingredient for an alcoholic beverage; (2) as a medicinal herb, mainly in infusions and decoctions; and (3) its essential oil as a raw material for perfumery (Fig. 6.7).

1) As an ingredient for an alcoholic beverage: *A. boronioides* can be found in a product called "Estepvka", made in El Calafate, Santa Cruz. The labeling of the product shows that its ingredients are water, alcohol and paramela. In addition, it is specified that the alcoholic concentration is 40% (http://www.latiendagourmet.com.ar/bebidas/espirituosas/vodka-estepvka/).
2) As a medicinal herb: there is fragmentary information about the volumes used for commercial sales, the existence of collection centers, and the number of marketing intermediaries involved. Empirical works carried out in the city of S.C. Bariloche show that this plant is mainly commercialized dried, sometimes fresh, and in very variable quantities in bulk, including branches, leaves and flowers. The main sales destinations are Patagonian herbalists, pharmacies and houses selling naturistic products, where the product is fractionated in the selling points (Cuassolo et al. 2010). *Adesmia boronoides* appears as one of the most commercialized native medicinal plants in the urban centers of the region (Cuassolo 2010).
3) Regarding its use as an essential oil, it has been used in perfumery since 2005, and to this purpose it is marketed and exported. The extraction and harvesting of the material comes only from natural populations. According to unverified sources, the amount of raw material would add to more than 300 tons to date. This material is processed solely by the distillation plants. There are audio-visual records of the recollection in Lago Buenos Aires, Santa Cruz province, where weekly truck shipments to the city of Esquel are mentioned (https://www.youtube.com/watch?v=mxSeFGrs30U).

In Chile, Paramela is advertised and sold as the "mapucheviagra": a herbal preparation called Palwen that increases sexual vigor (https://www.iucn.org/node/16897).

In 2008, the Natura cosmetic company started commercializing a perfume with a fragrance containing essential oil of paramela. The product is called "Amor América", and it was inspired by plants from the Andes and Patagonia, including not only the paramela but also the *palosanto* of Ecuador (*Bursera graveolens* (Kunth) Triana & Planch.).

Fig. 6.7 Alcoholic beverage and perfume with *A. boronioides* as an ingredient. Photograph by González S.B.

It is worth mentioning that the species has begun to be sold as ornamental in some regional fairs and nurseries of Patagonia because of the beauty of its flowers and its perennial character.

Domestication experiences and propagation

Background analysis shows that *A. boronioides* arouses much interest in companies on a commercial scale, and that the material is obtained only from natural populations.

Germination studies developed by the National University of Patagonia S.J.B. (UNPSJB), INTA and the National University of Río Negro (UNRN) made it possible to identify effective and practical methods for propagation. In order to successfully germinate *A. boronioides,* it is necessary to break the physical barrier using treatments that weaken the seed coat. From seeds collected in natural populations, pre-germination soaking treatments at 80°C yielded 85% germination success (González et al. 2009), and pre-scarification yielded 83.7% germination success (Mazzoni et al. 2014).

These experiments and results suggest that it would be feasible to produce large-scale seedlings to establish future crops as a productive alternative for the region.

Container cultivation was evaluated by the UNRN Tecnicatura en Viveros (Plant Nursery Technicature). They evaluated the production of 120 plants grown from seeds in one-liter containers with a substrate mixture of volcanic ash, peat and soil (Sánchez and Riat 2012). This plant material was transplanted in 2015 to a nursery on outdoor soil, located at INTA Bariloche to continue for future evaluations (Fig. 6.8).

Additionally, this exploratory cultivation test generated vegetal material that was harvested and is being characterized chemically in relation to its contents of essential oils in the UNPSJB. In this way the domestication studies carried out in the region try to make technical contributions that allow us to think about productive alternatives, reduce the collection pressure of the environment and generate products of commercial quality (Mazzoni et al. 2016, Contardi et al. 2016a, 2016b).

Fig. 6.8 Natural populations and stonemasons with specimens obtained from seeds of that origin. Photograph by González, S.B. and Mazzoni, A.

Conclusions

The studies presented in this chapter confirm the long history of use that *A. boronioides* has in the Mapuche-Tehuelche communities, as an aromatic and medicinal plant. Consistent with the commitment made by Argentina to the Convention on Biological Diversity and the Nagoya Protocol, it is necessary to develop regulations to control and protect the access to the traditional knowledge associated with this plant.

In relation to the use of paramela for human consumption, the results of the research are promising. To date, no evidence of toxicity or allergenicity has been found in traditional forms of use in terms of preparation and dosage. However, there are no studies on high-dose and long-term use safety. There are chemical studies that show variability in the chemical composition (qualitative and quantitative)

of the essential oil and in the content of phenols in different populations of *A. boronioides* throughout Patagonia. This means that in order to be able to recommend its consumption, the homogeneous quality of the chemical composition of the plants used for the production of beverages must be ensured. In turn, it would be important to develop systems for quality control and authentication of the raw material used as an additive in edible products.

Studies showed that propagation of *A. boronioides* was possible from seeds. The plants produced could be used to establish field crops in the future, and not rely on natural populations as the only alternative for obtaining plant material intended for the production of commercial products.

Finally, in order to fully understand the current situation of this species and all its potential uses it is necessary to adopt a multicultural and transdisciplinary approach. This approach should bring together the academy perspective with that of the local communities, who have been the main heirs and makers of this valuable Patagonian heritage.

Acknowledgements

The authors would like to acknowledge the financial support received through the projects: C. y T. N° 005/09, C.y T. N° 1039 UNPSJB, PIP 0466 UNCOMA and Professor Cristina Zuppa.

References

Agnese, A.M., Juliani, H.R. and Cabrera, J.L. 1989. Estudio fitoquímico en especies del género *Adesmia* (Fabaceae). An. Asoc. Quím. Argent. 77(4) 287–291.

Agnese, A.M. 1993. Estudio fitoquímico de especies del género *Adesmia* (Leguminosae). Tesis Doctoral de la Universidad Nacional de Córdoba 163 pp.

Bandoni, A. 2000. Los Recursos Vegetales Aromáticos en Latinoamérica, su aprovechamiento industrial para la producción de aromas y sabores. EDULP. La Plata 410 pp.

Barthelemy, D., Brion, C. and Puntieri, J. 2008. Plantas. Patagonia. Vázquez Mazzini Ed. Buenos Aires 239 pp.

Blengini, A., Muñoz, A.V., Artola, S., Gamarra, K. and Freile, M.L. 2016. Evaluación de la actividad antimicrobiana de Paramela (*Adesmia boronioides* Hook. f.). Dominguezia 32(2): 25.

Burkart, A.E. 1967. Sinopsis del género sudamericano de Leguminosas *Adesmia* D.C. Darwiniana 14: 463–468.

Burkart, A.E. 1984. *Adesmia*. En M.N. Correa (ed.). Flora Patagonica, Leguminosae. Col. Cient. INTA 4b: 92–161.

Campos, V.M, Cárdenas, E.M. and San Martín Saldias, C. 1997. Herbolaria médica de Chile. Diagnóstico de su estado actual y perspectivas futuras para la medicina oficial chilena. Ministerio de Salud, Santiago.

Castillo, L. and Ladio, A.H. 2014. Importancia del recurso vegetal como fuente de remedios caseros de uso veterinario para los pobladores rurales de Sierra Rosada, Sierra Ventana y El Escorial (Chubut). XXVI Reunión Argentina de Ecología. RAE 2014. Comodoro Rivadavia.

Cerda-García-Rojas, C. M., Bucio, A., González, S.B., García Gutiérrez, H. and Joseph-Nathan, P. 2015. Absolute configuration of esquelane derivatives from *Adesmia boronioides* by vibrational circular dichroism. Tetrahedron-Asymmetr. 26: 136–140.

Ciampagna, M.L. and Capparelli, A. 2012. Historia de uso de las plantas por parte de las poblaciones que habitaron la Patagonia Continental Argentina. Rev. Arqueol. 6: 45–75.

Contardi, L.T., Silva Sofrás, F.M. and González, S.B. 2016a. Paramela, una especie nativa de la Patagonia: análisis de frutos y semillas. Rev. Patag. For. – AÑO XXI, N°1, Junio 2016.

Contardi, L.T., Silva Sofrás, F.M. and González, S.B. 2016b. Reproducción *de Adesmia boronioides,* especie aromática y medicinal actualmente cosechada de poblaciones naturales en Patagonia. Rev. Nat. Patagon. 9: 50–60.

Conticello, L., Gandullo, R., Bustamante, A. and Tartaglia, C. 1997. El uso de plantas medicinales por la comunidad Mapuche de San Martín de los Andes, provincia de Neuquén (Argentina). Parodiana 10(1-2): 165–180.

Cuassolo, F., Ladio, A.H. and Ezcurra, C. 2010. Aspectos de la comercialización y control de calidad de las plantas medicinales más vendidas en una comunidad urbana del NO de la Patagonia Argentina. Bol. Latinoam. Caribe Plant. Med. Aromat. 9(3): 166–176.

de Araújo A. 1976. The relationship between altitude and cyanogenesis in white clover (*Trifolium repens* L.). Heredity 37(2): 291–293.

Delfino, M., Monelos, L., Peri, P. and Buffa, L. 2009. A new species of *Aphis* L (Hemiptera: Aphididae) from South Patagonia. Neotrop. Entomol. 38(3): 366–369.

Estomba, D., Ladio, A.H. and Lozada, M. 2006. Medicinal wild plant knowledge and gathering patterns in a Mapuche community from North-Western Patagonia. J. Ethnopharmacol. 103: 109–119.

Estomba, D., Mattes Fernández, H. and Stella, A.M. 2010. Antioxidantes y porfirinas de *Adesmia boronioides, Larrea divaricata* y *Atriplex lampa* cultivadas *in vitro* Rev. FCA UNCuyo 42(2): 135–142.

Eyssartier, C., Ladio, A. and Lozada, M. 2009. Medicinal use of plant species cultivated in a semi-rural community of the patagonian steppe. Bol. Latinoam. Caribe Plant. Med. Aromat. 8(2): 67–76.

Eyssartier, C., Ladio, A.H. and Lozada, M. 2011a. Traditional horticultural knowledge change in a rural population of the Patagonian steppe. J. Arid Environ. 75: 78–86.

Eyssartier, C., Ladio, A.H. and Lozada, M. 2011b. Horticultural and gathering practices complement each other: a case study in a rural population of Northwestern Patagonia. Ecol. Food Nutr. 50: 429–451.

Eyssartier, C., Ladio, A.H. and Lozada, M. 2013. Traditional horticultural and gathering practices in two semi-rural populations of Northwestern Patagonia: resilience and embodied know-how. J. Arid. Environ. 97: 18–25.

Faini, F., M. Castillo, R. Torres, G. DelleMonache and Gacs-Baitz, E. 1995. Malabaricane triterpene glucosides from *Adesmia aconcaguensis*. Phytochemistry 40(3): 885–890.

Forcone, A. and Muñoz, M. 2009. Floración de las especies de interés apícola en el Noroeste de Santa Cruz, Argentina. Bol. Soc. Argent. Bot. 44(3-4): 393–403.

Gastaldi B., Assef, Y., Sofrás, F., Catalán, C. and González, S. 2016. Actividad antioxidante y toxicidad frente a *Artemia salina* de infusiones de nueve especies aromáticas del noroeste de la Patagonia. Dominguezia 32(2): 39.

Gastaldi, B., Assef, Y., van Baren, C., Di Leo Lira, P., Retta, D., Bandoni, A. and González, S. 2016. Antioxidant activity in teas, tinctures and essential oils of native species from Patagonia Argentina. Rev. Cub. Pl. Med. 21(1): 51–62

González, S.B. 2005. *Adesmia boronioides* Hook. f.: una especie aromática y medicinal nativa de la Patagonia. Nat. Patagon. 2(1): 85–91.

González, S.B. and Molares, S. 2004. Plantas medicinales utilizadas en comunidades rurales del Chubut, Patagonia Argentina. S. Bol. Latinoam. Caribe Plant. Med. Aromat. 3(3): 58–62.

González, S.B., Gastaldi, B., Silva Sofrás, F., van Baren, C., Di Leo Lira, P., Retta, D. and Bandoni, A. 2016. Diversidad química en una especie aromática y medicinal endémica de Patagonia. Dominguezia 32(2): 89–90.

González, S.B. 2002. Tesis Doctoral FFyB, UBA. Estudio de *Adesmia boronioides* Hook.*f.*, una especie aromática y medicinal nativa de la Patagonia. Doctorado en el área Productos Naturales, 219 pp.

González, S.B., Bandoni, A.L., van Baren, C., Di Leo Lira, P., Cerda-García-Rojas, C.M. and Joseph-Nathan, P. 2004. The Essential Oil of the Aerial Parts of *Adesmia boronioides* Hook. *f.* J. Essent. Oil Res. 16: 513–516.

González, S.B., Bandoni, A.L., van Baren, C., Di Leo Lira, P., Cerda-García-Rojas, C.M. and Joseph-Nathan, P. 2002. Structure, conformation and absolute configuration of novel bisnorsesquiterpenes from the *Adesmia boronioides* essential oil. Tetrahedron, 58: 3065–3071.

González, S.B., Pasquini, N. and Contardi, L. 2009. Calidad de semillas y producción de plantas de *Adesmia boronioides* Hook. f., especie aromática y medicinal nativa de la Patagonia. Proyecto de Ciencia y Técnica de la UNPSJB sede Esquel. CAFCN N° 374/08, Resol C.S. N° 101/08.

González, S.B., Houghton, P. and Hoult, J. 2003.The activity against leukocyte eicosanoid generation of essential oil and polar fractions of *Adesmiaboronioides* Hook. f. Phytother. Res. 17: 290–293.

González, S.B., Gallardo, C. and Guerra, P. 2005. Etnobotánica de plantas medicinales en comunidades rurales de la Provincia del Chubut, Patagonia Argentina. Libro de Resúmenes V Reunión de la Sociedad Latinoamericana de Fitoquímica, Montevideo, 28 Noviembre al 2 de Diciembre de 2005, pág. 102.

González, S.B., Gallardo, C., Recalde, J. and Guerra, P. 2005. Talleres etnobotánicos en comunidades del NO del Chubut. Actas de las XXX Jornadas Argentinas de Botánica, Rosario, Argentina, 6 al 10 de Noviembre de 2005, pág. 83.

González, S.B., Guerra, P., Gastaldi, B., van Baren, C., Di Leo Lira, P. and Bandoni, A. 2014. Avances en el estudio de plantas aromáticas nativas y naturalizadas en la Patagonia Argentina. Variabilidad y constancia en la composición de sus aceites esenciales. Lilloa 52 (Suplemento): IV Jornadas Nacionales de Plantas Aromáticas Nativas y sus Aceites Esenciales, 37–42.

Green, L. and Ferreyra, M. 2011. Flores de la Estepa patagónica. Vázquez Mazzini Ed. Buenos Aires 286 pp.

Guignard, L. 1906 "Le Haricot à acide cyanhydrique ("Phaseolus lunatus acide cy anhydrique ("*Phaseolus lunatus*" L.), étude historique, botanique et chimique, nouveau procédé pour déceler l''acidecyanhydriquel ''acide cy anhydrique" Rev. Viticulture.

Igon, P., Ladio, A.H. and Lozada, M. 2006. Plantas Medicinales utilizadas en las comunidades de Villa Traful y Cuyín Manzano. UNCOMA. Ed. Imaginaria. 67 pp.

Kakes, P. 1990. Properties and functions of the cyanogenic system in higher plants. Euphytica 48: 23–43.

Kutschker, A., Menoyo, H. and Hechem, V. 2002. Plantas medicinales de uso popular en comunidades del oeste del Chubut. INTA-Universidad Nacional de la Patagonia S.J.B.-GTZ, Bariloche 139 pp.

Ladio, A.H. 2006. Gathering of wild plant foods with medicinal use in a mapuche community of NW patagonia. pp. 297–321. *In*: Pieroni, Andrea and Lisa Price (eds.). Eating and Healing: Explorations of Traditional Food as Medicines. Haworth Press. USA.

Ladio, A.H. and Molares, S. 2014. El paisaje patagónico y su gente. Capítulo 9. *In*: Raffaele, E., de Torres Curth, M., Morales , C.L. and Kitzberger, T. (eds.). Ecología e historia natural de la Patagonia Andina: Un cuarto de siglo de investigación en biogeografía, ecología y conservación. Ed. Fund. Azara, C.A. de Buenos Aires, 256 pp.

Ladio, A.H. and Molares, S. 2017. Capítulo 29. Etnoconservacionismo y prácticas locales en Patagonia: avances y perspectivas. pp. 649–672. *In*: Casas, A., Torres-Guevara, J. and y Parra, F. (eds.). Domesticación en el Continente Americano. Historia y perspectivas del manejo de recursos genéticos en el Nuevo Mundo. Universidad Agraria La Molina. IIES, Lima, Perú. ISBN 978-607-02-9334-4.

Ladio, A.H. 2006. Etnobotánica en la Patagonia: conocimiento local e importancia actual de los recursos vegetales en las comunidades rurales. ACCB 18: 13–29.

Ladio, A.H., Lozada, M. and Weigandt, M. 2007.Comparison of traditional wild plants use between two Mapuche communities inhabiting arid and forest environments in Patagonia, Argentina. J. Arid Environ. 69: 695–715.

Lukas G., 1964. The constitution of two new sesquiterpenic ketones. Furopelargones A and B. Tetrahedron. 20: 1789–1801.

Martínez-Crovetto, R. 1980. Apuntes sobre la vegetación de los alrededores del Lago Cholila. Publicación Técnica Nº 1, FCA UNNE, Corrientes, pp. 1–22.

Mazzoni, A., Sánchez, G., Riat, M. and Hagiwara, J. 2014. Propagación de *Adesmia boronioides* Hook. f., planta nativa medicinal de la Patagonia Argentina. Libro de resúmenes y poster presentado al XXXVII Congreso Argentino de Horticultura (ASAHO). Mendoza.

Mazzoni, A., Sánchez, G., Riat, M. and Hagiwara, J. 2016. Propagación de *Adesmia boronioides* Hook. f., planta nativa medicinal de la Patagonia Argentina. Presentación oral: Experiencias de Domesticación. V Jornadas Nacionales de plantas aromáticas nativas y sus aceites esenciales y Iº Jornadas Nacionales de plantas medicinales nativas. Esquel. Dominguezia 32(2): 70.

Molares, S. and Ladio, A.H. 2008. Plantas medicinales en una comunidad Mapuche del NO de la Patagonia Argentina: clasificación y percepciones organolépticas relacionadas con su valoración. Bol. Latinoam. Caribe Plant. Med. Aromat. 7(3): 149–155.

Molares, S. and Ladio, A.H. 2009. Ethnobotanical review of the Medicinal Mapuche Flora: use patterns on a regional scale. J. Ethnopharmacol. 122: 251–260.

Molares, S. and Ladio, A.H. 2012a. Mapuche perceptions and conservation of Andean *Nothofagus* forests and their medicinal plants: A case study from a rural community in Patagonia, Argentina. Biodivers. Conserv. 21(4): 1079–1093.

Molares, S. and Ladio, A.H. 2012b. The usefulness of edible and medicinal Fabaceae in Argentine and Chilean Patagonia: environmental availability and other sources of supply. Evid.-Based Compl. Alt. Article https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3238373/pdf/ECAM2012-901918.pdf.

Molares, S. and Ladio, A.H. 2014. Medicinal plants in the cultural landscape of a Mapuche-Tehuelche community in arid Argentine Patagonia: an eco-sensorial approach. J. Ethnobiol. Ethnomed. 10: 61.

Mongelli E., Coussio, J. and Ciccia, G. 1995. Estudio de toxicidad aguda de plantas medicinales argentinas mediante el bioensayo de *Artemia salina* Lech. Dominguezia 12: 35–42.

Montes, A.L. and Peltz, L. 1963. Esencias de plantas aromáticas del Parque Nacional Nahuel Huapi y sus aledaños. 2. *Adesmia boronioides* Hooker, o paramela. An. Soc. Cient. Argent. 175: 91–101.

Montes, O. Montes, M. and T . Wikomirsky. 2001. Plantas Medicinales de Uso en Chile. Química y Farmacología. Editorial Universitaria, S.A. Santiago, Chile.

Muñoz, O., Montes, M. and Wilkomirsky, T. 2001 Plantas medicinales de uso en Chile. Química y Farmacología. Ed. Universitaria. Santiago. 205 pp.

Nájera, M.T., Spegazzini, E.D., Castro, M.T., Guerra, P. and González, S. 2000. Anatomía de *Adesmia boronioides* Hook. f. (Leguminosae - Papilionoideae). Acta Farm. Bonaer. 19(4): 245–250.

Ochoa, J. 2008. Aspectos ecológicos y socioculturales asociados al uso de plantas silvestres en una población rural del Noroeste de la Patagonia.Tesis de Licenciatura. Universidad Nacional del Comahue, S.C. de Bariloche, Argentina.

Ochoa, J., Ladio, A.H. and Lozada, M. 2010. Uso de recursos herbolarios entre mapuches y criollos de la comunidad campesina de Arroyo Las Minas (Río Negro, Patagonia Argentina). Bol. Latinoam. Caribe Plant. Med. Aromat. 9(4): 269–276.

Ochoa, J. 2015.Uso de plantas silvestres con órganos de almacenamiento subterráneos comestibles en Patagonia: perspectivas etnoecológicas. Tesis para optar al grado de Doctor en Biología. Universidad Nacional del Comahue. S.C. de Bariloche (Río Negro). 250 pp.

Pérez, O. and Lazo, F. 2010. Ensayo de *Artemia*: útil herramienta de trabajo para ecotoxicólogos y químicos de productos naturales. Rev. Protección Veg. 22(1): 34–43.

Richards, A.J. and Fletcher, A. 2002. The effects of altitude, aspect, grazing and time on the proportion of cyanogenics in neighbouring populations of *Trifolium repens* L. (white clover). Heredity 88: 432–436.

Richeri, M., Ladio, A.H. and Beeskow, A.M. 2013. Conocimiento tradicional y autosuficiencia: la herbolaria rural en la Meseta Central del Chubut (Argentina). Bol. Latinoam. Caribe Plant. Med. Aromat. 12(1): 44–58.

Richeri, M. 2016. Evaluación de los reservorios etnobotánicos en las poblaciones rurales del centro norte de la Provincia del Chubut. Tesis para optar al grado de Doctor en Biología. Universidad Nacional del Comahue. S.C. de Bariloche (Río Negro). 170 pp.

Richeri, M., Cardoso, M.B. and Ladio, A.H. 2013. Soluciones locales y flexibilidad en el conocimiento ecológico tradicional frente a procesos de cambio ambiental: estudios de caso en Patagonia. Ecol. Aust. 23: 184–193.

Sánchez, G. and Riat, M. 2012. Estudio de *Adesmia boronioides* Hook. f. "Paramela" (Fabáceas). Un acercamiento para su reproducción y manejo en viveros. Informe técnico. Carrera Tecnicatura en Viveros. UNRN. Sede Andina. San Carlos de Bariloche. Río Negro.

Silva Sofrás, F., Gastaldi, B. and González, S. 2016. Compuestos fenólicos de *Adesmia boronioides* Hook f.: Análisis de su variabilidad en dos estaciones diferentes. Dominguezia (32)2: 54.

Toledo, C. and Kutschker, A. 2012. Plantas medicinales en el Parque Nacional Los Alerces, Chubut, Patagonia Argentina. Bol. Soc. Argent. Bot. 47(3-4): 461–470.

Ulibarri, E. and Burkart, A. 2000. Sinopsis de las especies de *Adesmia* (Leguminosae-Adesmiae) de la Argentina. Darwiniana 38 (1-2): 59–126.

Ulibarri, E.A. and Simpson, B.B. 2010. Una nueva especie de *Adesmia* (*Leguminosae, Papilionoideae*) para la Argentina. Darwiniana 48(2): 204–207.

Vetter, J. 2000. Plant cyanogenic glycosids.Toxicon 38: 11–36.

Villagra, S., González, S., González Rojas, S., Garriz, N., Longarzo, A., Errecalde, J. and Zaidenberg, A. 2008. Inhibición *in vitro* de *Tripanosoma cruzi* por aceites esenciales. VIII Congreso Argentino de Protozoología y Enfermedades Parasitarias, 2 al 5 de Noviembre de 2008, Rosario, Santa Fe, Argentina. Rev. Med. Rosario 74 (SUPL).

Weigandt, M., Ladio, A.H. and Lozada, M. 2004. Plantas medicinales utilizadas en la comunidad Mapuche Curruhuinca. Secretaría de ExtensiónUniversitaria. UNCo. Ed. Imaginaria. Bariloche 73 pp.

7

The Genus *Vismia*: Geographical Distribution, Chemical Composition and Recent Biological Studies

*Janne Rojas** and *Alexis Buitrago*

Introduction

Plants usage is considered an ancient practice where a religious-magical touch also took place for the treatment of several human pathologies. Despite the modern practice of medicine and the use of prescription drugs, nowdays, there is an increased interest to discover new medicines from natural sources (Rates 2001). Thus, plants have been under investigation to determine biological activities that might be useful as a natural alternative for the treatment of human diseases.

Hypericaceae family comprises nine genera: *Cratoxylum* Blume, *Eliea* Cambess, *Harungana* Lamarck, *Hypericum* L., *Lianthus* N. Robson, *Santomasia* N. Robson, *Thornea* Breedlove & Mc Clintock, *Triadenum* Rafinesque and *Vismia* Vand (Crocketta and Robinson 2011). *Vismia* genus is composed of approximately 55 species, distributed in tropical and subtropical regions of Central and South America, although there are some species reported in Africa (Hussain et al. 2012). Phytochemical studies conducted on different *Vismia* species have reported around 161 chemical compounds, the majority of these with aromatic structures, holding oxygenated functions such as anthrones, xanthones, anthraquinones, among others, which are biosynthetically generated by the acetate malonate pathway. Other type of components isolated from this genus comprises flavonoids, originated by the combination of acetate malonate-shikimate pathways and terpenes biosynthetized by mevalonic acid pathway (Dewick 2002, Vizcaya et al. 2014).

Regarding biological properties, many of *Vismia* species have been used in traditional medicine to treat ulcerations, fungus, herpes, as laxatives and to treat high fever (Vizcaya et al. 2014, Buitrago et al. 2016). Furthermore, isolated compounds have demonstrated antimicrobial, antioxidant, antinoniceptive, leishmanicide, trypanomicide, cytotoxic, among other activities (Mbwambo et al. 2004, Salas et al. 2007a, Salas et al. 2007b, Tala et al. 2011).

This chapter aims to summarize some taxonomic features, geographical distribution, chemical composition and biological activitites, reported within the last 20 years for different *Vismia* species, in order to give authentic information to readers that might be useful for further investigations.

Organic Biomolecular Research Group, Research Institute, Faculty of Pharmacy and Bioanalysis, University of Los Andes, Mérida 5101, Venezuela.
* Corresponding author: janner@ula.ve

Significant botanical features

Hypericaceae family

Hypericaceae family belongs to order Malpigphiales and subclass Rosidae. It used to be included in to Clusiaceae family (Guttiferae family, *sensu lato*) but it was separated due to different phylogenetic and morphological features (Stevens 2007, Angiosperm Phylogeny Group 2009). This family comprises three tribes; Cratoxyleae, Hypericeae and Vismieae; with around 560 species distributed in nine genera: *Cratoxylum* Blume, *Eliea* Cambess, *Harungana* Lamarck, *Hypericum* Linneo, *Lianthus* N. Robson, *Santomasia* N. Robson, *Thornea* Breedlove & McClintock, *Triadenum* Rafinesque and *Vismia* Vand. (Stevens 2007, Angiosperm Phylogeny Group 2009, Crocketta et al. 2011).

Regarding botanical features, this family occurs as annual or perennial herbs, shrubs, and seldom trees, holding single opposite or sessile whorled leaves, sometimes dotted with tiny black or translucent spots. Limb with sub-parallel or cross-linked venation, yellow transparent glands turning dark yellow at surface and borders. Bisexual actinomorph flowers, yellow, orange or red color perianth, frequently with the presence of red streaks, arranged in branched inflorescences, four to five sepals and four to five petals; being attached below the ovary. Stamens have long filaments and sometimes are fused together. Three to five styles are usually fused at the base. Fruits are present as green dehiscent capsules turning to brown at maturity where these open to release the seeds (Nürk et al. 2013).

Vismia genus

Vismia genus belongs to the Vismieae tribe, composed of aproximately 55 species that grow between temperate to tropical climates. It is represented by trees and shrubs around 1 to 15 m of heigh. A highlight feature of this genus is the orange latex that exudes when a cut occurs in any part of the plant. It possesses ferruginous induments of single to starry hairs, opposite leaves with diferent forms such as lanceolate, oblong-lanceolate and oblong-ovate. It exhibits terminal panicles, with pentamerous flowers of numerous stamens grouped in fascicules; fruits as capsules or berries with persistant chalice (Álvarez et al. 2009).

Geographical distribution of Vismia genus

Species of *Vismia* genus are distributed mainly at Neotropic zones, from South of Mexico, passing through Central America till they reach North of Brazil, in South America. However, six species have been reported for the tropical areas of Africa. This occurrence, has been explained by the Gondwana super continent that existed at the Permic period (Botta et al. 1986, Stevens, 2007, Angiosperm Phylogeny Group 2009, Crocketta et al. 2011, Ruhfel et al. 2011). Table 7.1, describes the geographical distribution, synonymous and common names of different *Vismia* species (Hokche et al. 2008, "Trópicos org." 2015).

Secondary metabolites isolated from Vismia genus

Primary metabolism of plants involve different chemical processes such as photosynthesis, glycolysis, citric acid cycle, amino acid synthesis, among others, where carbohydrates, lipids and proteins take place to aid survival, growth and reproduction of plants, furthermore, Secondary Metabolites (SM) are designed as part of this process (Dewick 2002, Shilpa et al. 2010).

In this regard, SM are chemical compounds biosynthesized by plants that play important roles such as defense against herbivorous, pathogenic microorganisms, pollinators attraction and seed dispenser. However, humans have discovered that, such a variety of compounds may possess biological activities to treat a number of diseases (Goossens et al. 2003, Wink 2007).

There are three main biosynthetic pathways from where SM are designed; Shikimate that produces aromatic amino acids, phenylpropanoids, cinnamic acids, lignans, coumarins, flavonoids and stilbenes; mevalonate that generate terpenoids and steroids and acetate pathway that develops fatty acids and polyketides (Dewick 2002, Marcano and Hasegawa 2002).

Table 7.1 Worldwide geographical distribution of *Vismia* species (Hokche et al. 2008, "Trópicos org." 2015).

Especie	Synonymous	Common name	Country
Vismia acuminate (Lam.) Pers	*Hypericum acuminatum* Lam	--------	French Guyana
Vismia amazonica Ewan	*Vismia gracilis* Hieron	Pichirina Big leave	Bolivia, Brazil, Colombia, Guyana, Peru
Vismia angusta (Miq.)	*Caopia cordata* Rusby, *Hypericum reticulatum* Poir	Manchador, pichirina	Bolivia, Brazil, Colombia, French Guyana, Peru, Surinam, Venezuela
Vismia angustifolia Rusby	*Vismia falcata* Rusby, *Vismia laxiflora* Reichardt	--------	Venezuela
Vismia baccifera (L.) Triana & Planch	*Caopia baccifera* (L.) Kuntze, *Hypericum bacciferum* L., *Vismia dealbata* Kunth, *Vismia mexicana* Schltdl., *Vismia panamensis* Duchass & Walp	Carate, red carate, spearhead, achiotillo	Belice, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, El Salvador, French Guyana, Guatemala, Honduras, Mexico, Panama, Peru, Surinam, Venezuela
Vismia baccifera subsp. *dealbata* (Kunth) Ewan	*Caopia dealbata* (Kunth) Kuntze, *Vismia confertiflora* Spruce ex Reichardt, *Vismia dealbata* Kunth, *Vismia hamanii* S.F. Blake	Lancetillo, onotillo, spearhead	Brazil, Colombia, French Guyana, Surinam, Venezuela
Vismia baccifera subsp. *ferruginea* (Kunth) Ewan	*Caopia ferruginea* (Kunth) Kuntze, *Vismia cuspidata* Steud, *Vismia ferruginea* Kunth	Carate	Colombia, Venezuela
Vismia baccifera subsp. *subcuneata* (Huber) Ewan	*Vismia subcuneata* Huber	--------	Bolivia, Peru
Vismia bemerguii M.E. Berg	--------	--------	Brazil, Bolivia
Vismia billbergiana Beurl	*Caopia billbergiana* (Beurl.) Kuntze, *Vismia viridiflora* Duchass. ex Triana & Planch	Sangrillo	Belice, Colombia, Costa Rica, México, Nicaragua, Panama
Vismia boliviana Melch	--------	--------	Bolivia
Vismia brasiliensis Choisy	*Caopia brasiliensis* (Choisy) Kuntze, *Vismia brasiliensis* var. *lasiantha* Reich. in Martius, *Vismia laccifera* Mart	Pau-de-lacre, purga-de-vento	Brazil
Vismia buchtienii Ewan	*Vismia glaziovii* Ruhland, *Vismia gracilis* Hieron	Sangrito, puntelanza, pichirina blanca, bloodwood	Bolivia, Brazil, Peru, Venezuela
Vismia calvescens Gilg & Hieron	*Vismia lauriformis* (Lam.) Choisy	Lacre, lacre minchuba	Colombia
Vismia camparaguey Sprague & L. Riley	--------	--------	Belice, Guatemala, Honduras, Mexico
Vismia caparosa Kunth	*Vismia guianensis* (Aubl.) Choisy	--------	Venezuela
Vismia cavalcantii M.E. Berg	--------	--------	Suriname
Vismia cavanillesiana Cuatrec	--------	--------	Colombia, Ecuador
Vismia cayennensis (*Jacq.*) Pers	*Caopia acuminata* (Lam.) Kuntze, *Caopia cayennensis* (Jacq.) Kuntze, *Hypericum cayennense* Jacq, *Vismia floribunda* Sprague	Pichirina negra, Manchador, Lacre blanco, spearhead	Bolivia, Brazil, Colombia, Ecuador, French Guyana, Peru, Suriname, Trinidad and Tobago, Venezuela

Table 7.1 contd....

...Table 7.1 contd.

Especie	Synonymous	Common name	Country
Vismia cayennensis var. *sessilifolia* (Aubl.) M.E. Berg	*Vismia sessilifolia* (Aubl.) DC	--------	Guyana
Vismia cearensis Huber	--------	--------	Brazil
Vismia confertiflora Spruce ex Reichardt	*Caopia confertiflora* (Spruce ex Reichardt)	--------	Brazil, Colombia, Ecuador, Guyana
Vismia crassa (Rusby) S.F. Blake	*Caopia crassa* Rusby	--------	Bolivia
Vismia cuatrecasasii Ewan	--------	--------	Colombia
Vismia decipiens Schltdl. & Cham	*Vismia pentagyna* (Spreng.) Ewan	--------	Bolivia, Brazil, Venezuela
Vismia falcata Rusby	*Vismia angustifolia* Rusby	--------	Brazil, Trinidad, Tobago, Venezuela
Vismia glabra Ruiz & Pav	*Caopia glabra* (Ruiz & Pav.) Kuntze	--------	Bolivia, Brazil, Peru
Vismia glaziovii Ruhland	*Vismia buchtienii* Ewan	--------	Bolivia, Brazil
Vismia guaramirangae Huber	*Vismia reichardtiana* (Kuntze) Ewan	Capianga, lacre	Brazil
Vismia guianensis (Aubl.) Choisy	*Caopia guianensis* (Aubl.) A. Lyons, *Hypericum acuminatum* Lam., *Hypericum guianense* Aubl.	Uadama, punta de lanza lacre	Bolivia, Brazil, Colombia, French Guyana, Surinam, Venezuela
Vismia guianensis subsp. *persicoides* Ewan	--------	--------	Colombia
Vismia japurensis Reichardt	*Caopia japurensis* (Reichardt) Kuntze	Lacre, picharrinha, purga-de-vento	Brazil, Colombia, Guyana, Surinam, Venezuela
Vismia jefensis N. Robson	--------	Rastrojero, sangrito	Colombia, Panama
Vismia laevis Triana & Planch	*Caopia laevis* (Triana & Planch.) Kuntze	Carate, punta de lanza	Colombia, Venezuela
Vismia latifolia Kunth	*Vismia humboldtiana* Schltdl. & Cham	--------	Venezuela
Vismia latisepala N. Robson	--------	Sangrito, achioto	Panama
Vismia macrophylla Kunth	*Caopia macrophylla* (Kunth) Kuntze, *Vismia macrophylla* var. *glabrescens* Hochr	Sangrillo, sapidalle, carachero,sapigale, lacre hojiancho, lanzo, fierro lanzo, lacre, manchador	Belice, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guyana, Guyana, Guatemala, Panama, Surinam
Vismia mandar Hieron	*Caopia mandurr* Hieron	--------	Colombia, Ecuador
Vismia martiana Reichardt	*Caopia martiana* (Reichardt) Kuntze	--------	Brazil
Vismia micrantha Mart. ex A. St.-Hil	*Caopia micrantha* (Mart. ex A. St.-Hil.) Kuntze	--------	Brazil
Vismia minutiflora Ewan	--------	--------	Bolivia, Brazil, Colombia, Ecuador, Peru
Vismia obtusa Spruce ex Reichardt	*Caopia obtusa* (Spruce ex Reichardt) Kuntze	--------	Brazil, Colombia, Ecuador, Peru
Vismia orientalis Engl	--------	--------	Tanzania
Vismia ovalifolia Melch	--------	--------	Peru

Table 7.1 contd....

...Table 7.1 contd.

Especie	Synonymous	Common name	Country
Vismia panamensis Duchass. & Walp	*Caopia panamensis* (Duchass. & Walp.) Kuntze	--------	Colombia, Ecuador, Panama
Vismia parviflora Schltdl. & Cham	*Caopia parviflora* (Schltdl. & Cham.) Kuntze	--------	Brazil
Vismia pauciflora Milne-Redh	--------	--------	Tanzania
Vismia plicatifolia Hochr	*Caopia parvifolia* Rusby	--------	Bolivia
Vismia pozuzoensis Engl	*Vismia glabra* subsp. *pozuzoensis* (Engl.) Ewan	Pichirina	Bolivia, Brazil, Ecuador, Peru
Vismia ramuliflora Miq	*Vismia sessilifolia* (Aubl.) DC	--------	French Guyana, Guyana, Surinam
Vismia rubescens Oliv	--------	--------	Equatorial Guinea, Gabon
Vismia rusbyi Ewan	--------	--------	Bolivia, Botswana, Peru
Vismia sandwithii Ewan	--------	--------	Brazil, Ecuador, French Guyana, Guyana, Surinam
Vismia schultesii N. Robson	*Vismia tomentosa* Ruiz & Pav	--------	Bolivia, Brazil, Colombia, Ecuador, Peru
Vismia sprucei Prague	--------	--------	Bolivia, Brazil, Colombia, Ecuador, Peru
Vismia steyermarkii N. Robson	--------	--------	--------
Vismia urceolata Ewan	--------	--------	Colombia
Vismia viridiflora Duchass.ex Triana & Planch	*Caopia viridiflora* (Duchass. ex Triana & Planch.) Kuntze	--------	Colombia, Panama

In this regard, *Vismia* genus biosynthesizes a variety of SM, mainly through acetate and shikimate pathways, although, the mevalonate way is also used by this genus to produce terpene type components. The biosynthetic pathways used by plants for the production of their MS are described below, and some examples of isolated compounds from *Vismia* genus are also defined.

Acetate Malonate pathway

Polyketides comprises a large class of natural products with diverse structures derived from poly-β-keto chains formed by coupling of two molecules of acetyl-CoA via Claisen reactions, giving acetoacetyl-CoA. These compounds are fatty acids, polyacetylenes, prostaglandins, macrolide, antibiotics and many aromatic compounds, such as anthraquinones and tetracyclines. The biosynthesis involves initial carboxylation of acetyl-CoA to malonyl-CoA, a reaction involving ATP, CO_2 and biotin, a coenzyme acting as CO_2 carrier (Dewick, 2002, Marcano and Hasegawa, 2002).

The conversion of acetyl-CoA into malonyl-CoA increases the acidity of α-hydrogens, providing a better nucleophile for the Claisen condensation, thus, the carboxyl group introduced into malonyl-CoA is simultaneously lost by a decarboxylation reaction during the Claisen condensation. (Dewick 2002). Figure 7.1, illustrates part of the acetate malonate pathway from where many of anthraquinones, xanthones, anthrones and some other derivatives, found in *Vismia* species, are biosynthesized.

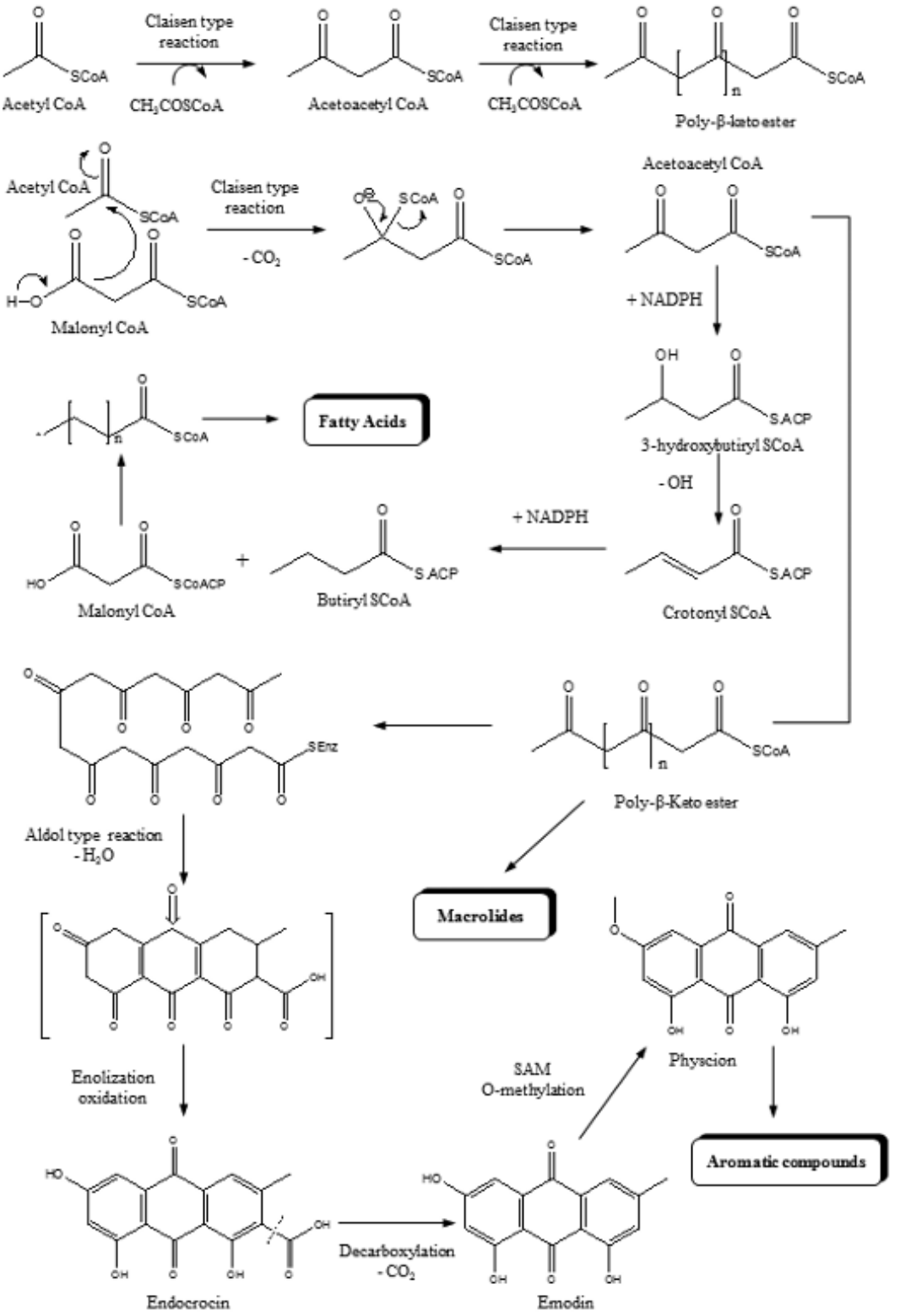


Fig. 7.1 Acetate malonate pathway (Dewick 2002, Marcano and Hasegawa 2002).

Shikimate pathway

This pathway is considered one of the most important ways, used by plants, to produce a variety of compounds, such as aromatic amino acids, benzoic acids, cinnamic acids, lignans, lignins, phenylpropenes, coumarins, flavonoids and stilbenes. It begins with a coupling of phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate to give 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP).

Elimination of phosphoric acid from **DAHP** followed by an intramolecular aldol reaction generates 3-dehydroquinic acid. Dehydration and reduction of 3-dehydroquinic acid leads to shikimic acid. A further molecule of PEP combines with shikimic acid 3-phosphate giving 3-enolpyruvylshikimic acid 3-phosphate (EPSP). 1,4-elimination of phosphoric acid leads to chorismic acid that is transformed into prephenic acid by the enzyme chorismate mutase. Decarboxylative aromatization of prephenic acid yields phenylpyruvic acid. Transamination of 4-hydroxyphenylpyruvic acid subsequently gives L-tyrosine and L-phenylalanine, precursors of a wide range of natural products (Dewick 2002). Figure 7.2, partially illustrates the shikimate pathway.

Vismia genus produces a majority of secondary metabolites through a combination of acetate and shikimate pathways exhibiting aromatic structures like anthrones, anthraquinones, xanthones, flavonoids, lignans, among others. Figures 7.3 to 7.19 show examples of isolated compounds from different *Vismia* species (Hussain et al. 2012, Vizcaya et al. 2012).

Mevalonate pathway

Terpenoids are considered a large and diverse family of natural products derived from isoprene units ($\mathbf{C_5}$), mainly joined through the head to the tail. These are classified, according to the number of isoprene units incorporated, as hemiterpenes ($\mathbf{C_5}$), monoterpenes ($\mathbf{C_{10}}$), sesquiterpenes ($\mathbf{C_{15}}$), diterpenes ($\mathbf{C_{20}}$), sesterterpenes ($\mathbf{C_{25}}$), triterpenes ($\mathbf{C_{30}}$) and tetraterpenes ($\mathbf{C_{40}}$) (Dewick 2002, Marcano and Hasegawa 2002).

The biosynthesis begins with formation of two reactive units, dimethylallyl diphosphate (**DMAPP**) and isopentenyl diphosphate **(IPP**), these are hemiterpene intermediates leading to more complex terpenoid structures. Combination of **DMAPP** and **IPP** yields geranyl diphosphate (**GPP**). The resulting compounds of this combination may be hydrocarbons, alcohols, aldehydes and esters. Addition of a further **IPP** unit to geranyl diphosphate leads to farnesyl diphosphate (**FPP**), a sesquiterpene precursor. Diterpenes arise from geranylgeranyl diphosphate (**GGPP**), which is formed by the addition of a further **IPP** molecule to farnesyl diphosphate in the same manner as described before. However, triterpenes are not formed by an extension of another **IPP** unit, instead, two molecules of farnesyl **IPP** are joined from tail to tail to yield the hydrocarbon squalene that is a precursor of triterpenes and steroids. Figure 7.20, illustrates the mevalonate pathway that produces the huge family of terpenoids and steroids (Dewick 2002, Marcano and Hasegawa 2002).

Studies carried out on different *Vismia* species have reported a number of terpenes, especially triterpenes, although, some sesquiterpenes have also been identified (Hussain et al. 2012, Vizcaya et al. 2012). Figures 7.21 to 7.23 show examples of sesquiterpenes and triterpenes isolated from several *Vismia* species.

Studies on different biological activities conducted on species of *Vismia* genus in the last twenty years

Plant extracts are considered an important source of secondary metabolites and are the starting point to the new drugs discovery with possible therapeutic activities. In this regard, phytochemical specialized laboratories have established preliminary biological screenings that allow obtaining rapid results, at low costs, to acknowledge if the extract under investigation has the required activity (Rahman et al. 2001, Marcano and Hasegawa 2002).

Furthermore, biological screening allows knowing the effect of heterogeneous samples such as extracts as well as pure isolated compounds under specific and controlled experimental conditions. Such studies are carried out *in vivo* (experimental animals, bacteria, fungus, among others) and *in vitro* (isolated

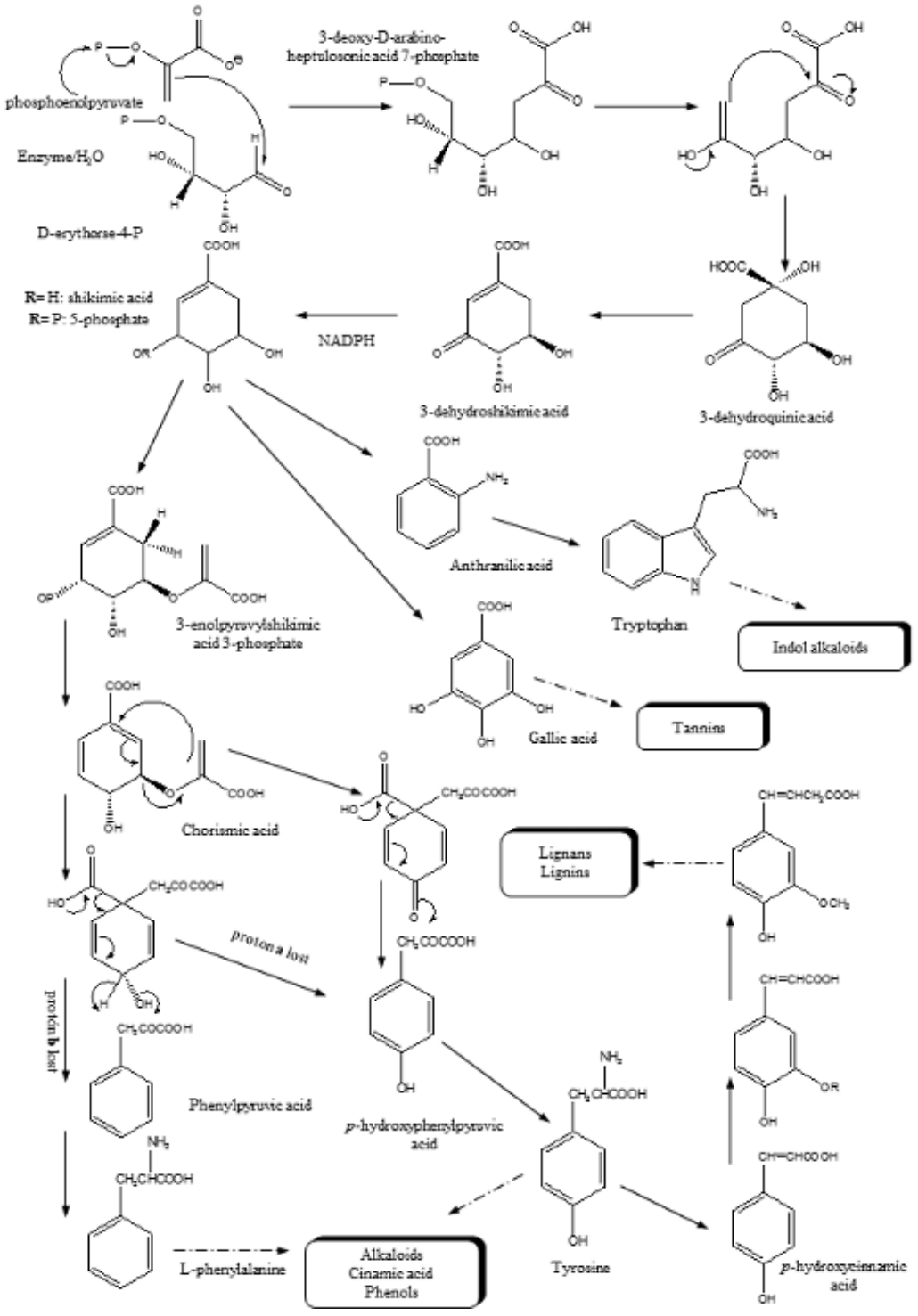


Fig. 7.2 Shikimate Pathway (Dewick 2002, Marcano and Hasegawa 2002).

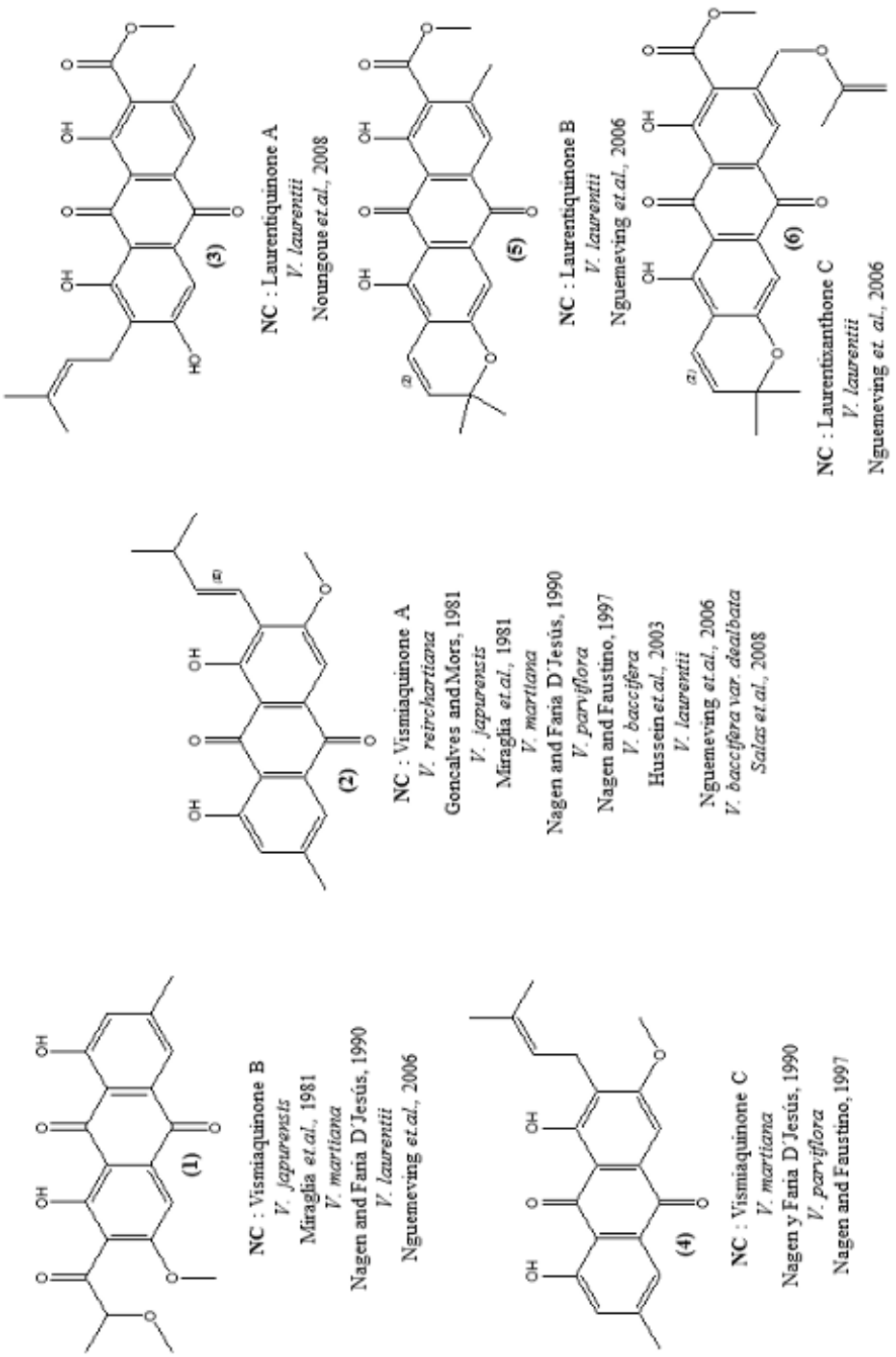
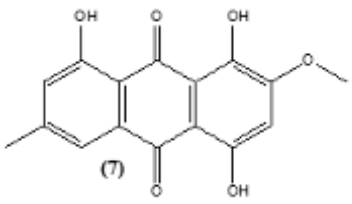
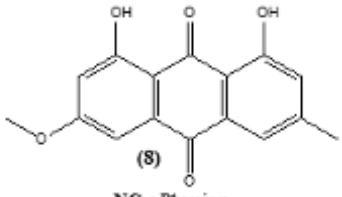


Fig. 7.3 Anthraquinones isolated from different species of *Vismia* genus.



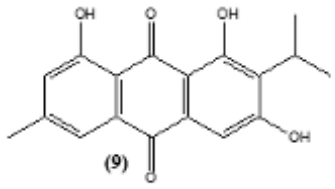
(7)

NC : Isoxanthrorin
V. laurentii
Noungoue *et.al.*, 2008



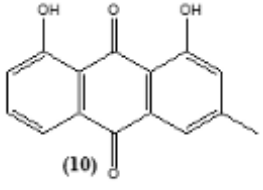
(8)

NC : Physion
V. martiana
Nagen and Faria D´Jesús, 1990
V. guaramirangae
Camelle *et.al.*, 1982
V. japurensis and V. mexicana
Pinheiro *et.al.*, 1984
V. rubescens
Schultes, 1993



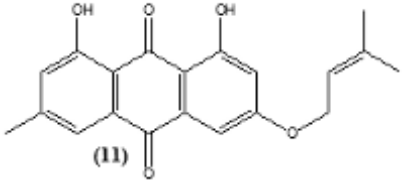
(9)

NC : 2-Isoprenyl-emodin
V. guaramirangae
Camelle *et.al.*, 1982.



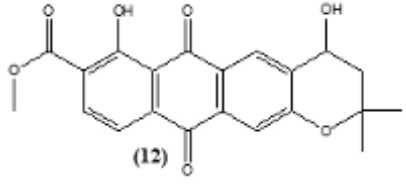
(10)

NC : Crysophanic acid
V. guaramirangae
Camelle *et.al.*, 1982
V. cayenensis
Pinheiro *et.al.*, 1984
V. martiana
Nagen and Faria D´Jesús, 1990
V. parviflora
Nagen and Faustino, 1997



(11)

NC : Madagascin
V. guaramirangae
Camelle *et.al.*, 1982
V. martiana
Nagen and Faria D´Jesús, 1990
V. parviflora
Nagen and Faustino, 1997



(12)

NC : Methyl-5,7-dihydroxy-2,2,9-trimethyl-6,11-dihydroxy-2–Hanthra [2,3-b] piran-8 -carboxylate
V. laurentii
Tchamo *et.al.*, 2008

Fig. 7.4 Anthraquinones isolated from different species of *Vismia* genus.

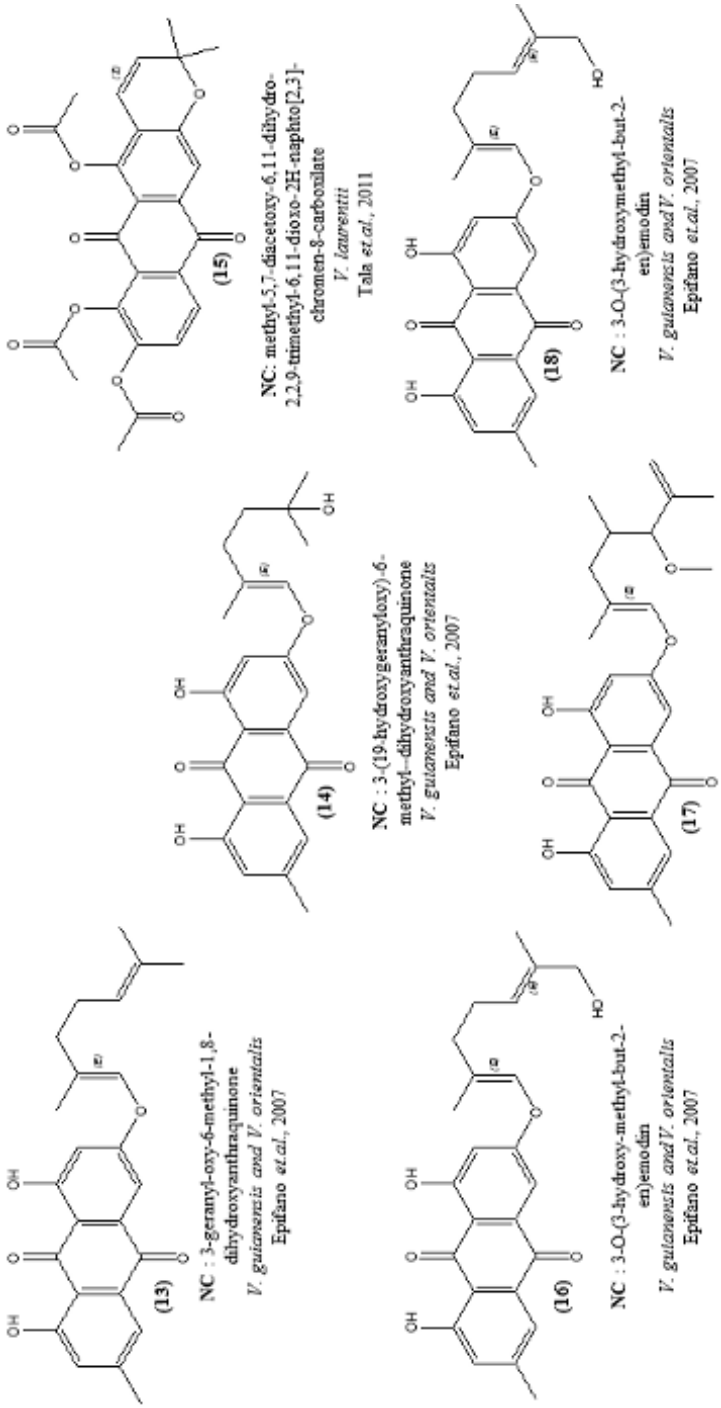
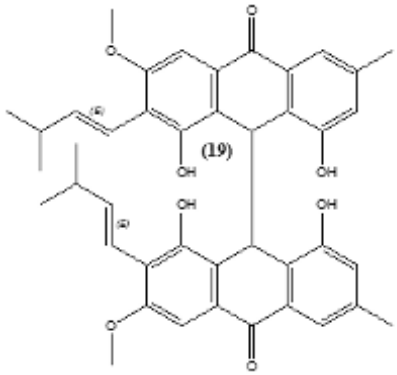
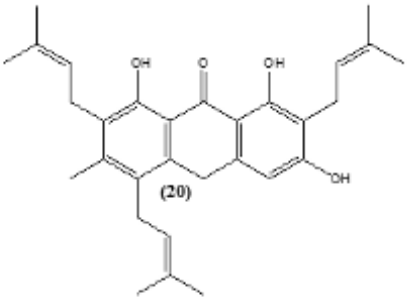


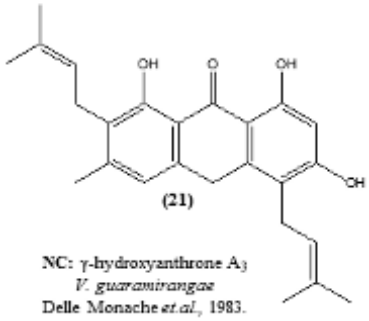
Fig. 7.5 Anthraquinones isolated from different species of *Vismia* genus.



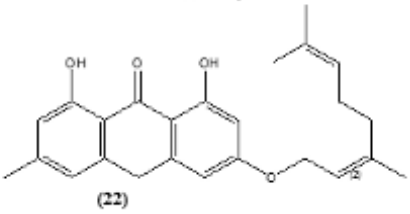
NC: 1,1',8,8'-tetrahydroxy-3,3'-dimethyl-6,6'-dimethoxy-7,7'-(trans-3-methyl-1-butenyl)-10,10'-bianthrone
V. martiana
Nagen and Faria, 1990



NC : Ferruanthrone
V. baccifera var. ferruginea
Delle Monache *et.al.*, 1979.
V. decipiens
Gonzales *et.al.*, 1980.

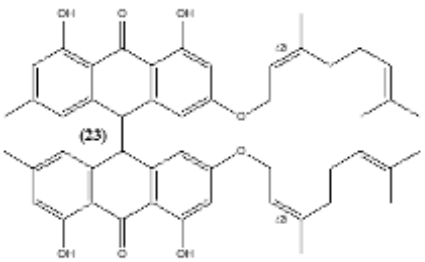


NC: γ-hydroxyanthrone A_3
V. guaramirangae
Delle Monache *et.al.*, 1983.

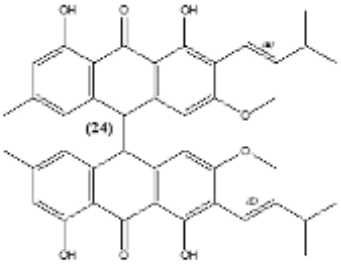


NC : 3-geranyloxy-6methyl-1,8-dihydroxyanthrone
V. laurentii
Nguemeving *et.al.*, 2006

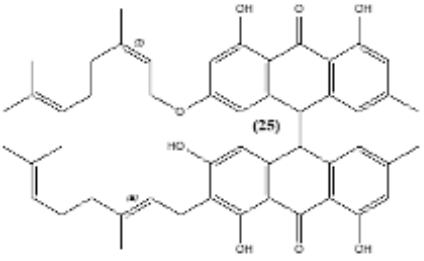
Fig. 7.6 Anthrones isolated from different species of *Vismia* genus.



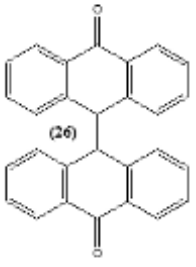
NC :Bianthrone A1
V. guineensis
Politi *et.al.*, 2004



NC : Bivismiaquinone
V. laurentii
Nguemeving *et.al.*, 2006



NC : Bianthrone A3a
V. guineensis
Politi *et.al.*, 2004



NC Dihydrodianthrone
V. magnoliaefolia
Nagen and Alves, 1991

Fig. 7.7 Anthrones isolated from different species of *Vismia* genus.

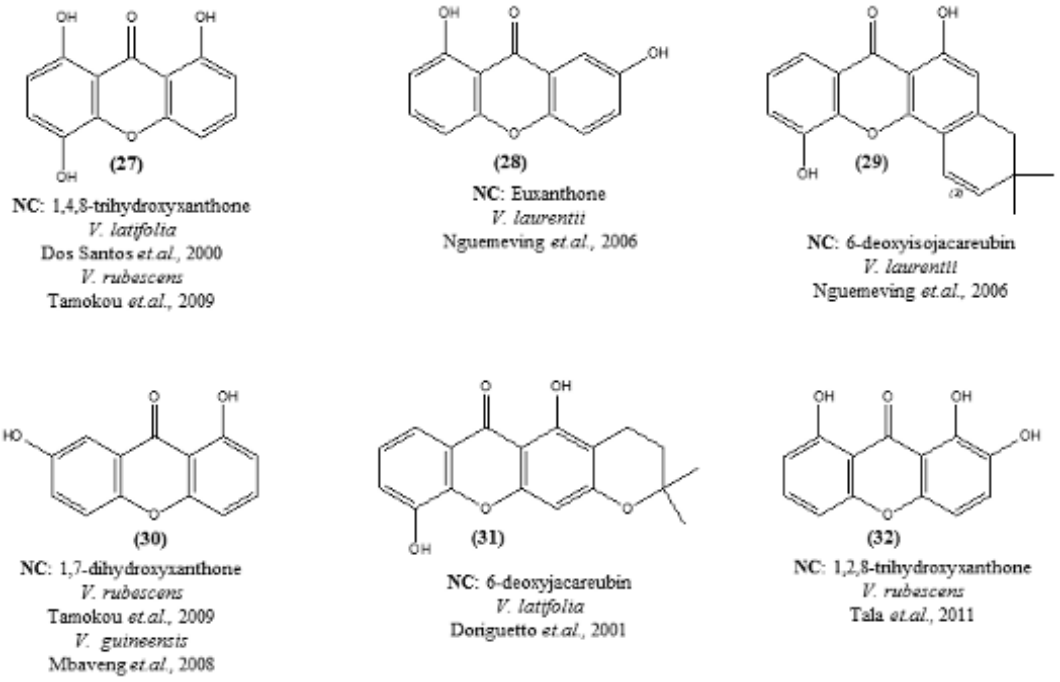


Fig. 7.8 **Xanthones isolated from different species of *Vismia* genus.**

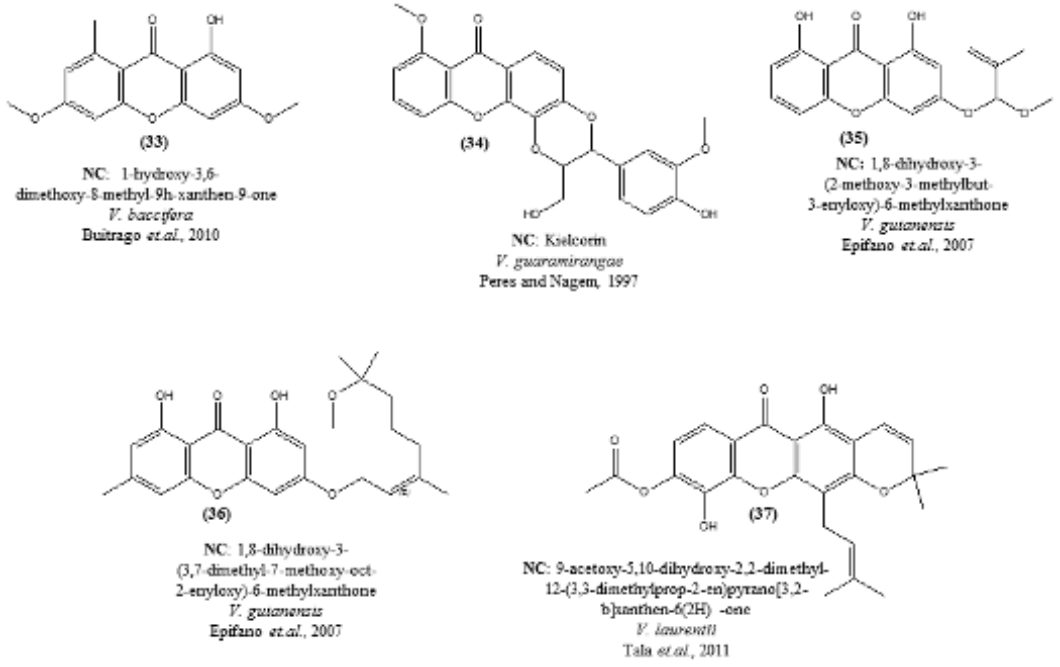


Fig. 7.9 Xanthones isolated from different species of *Vismia* genus.

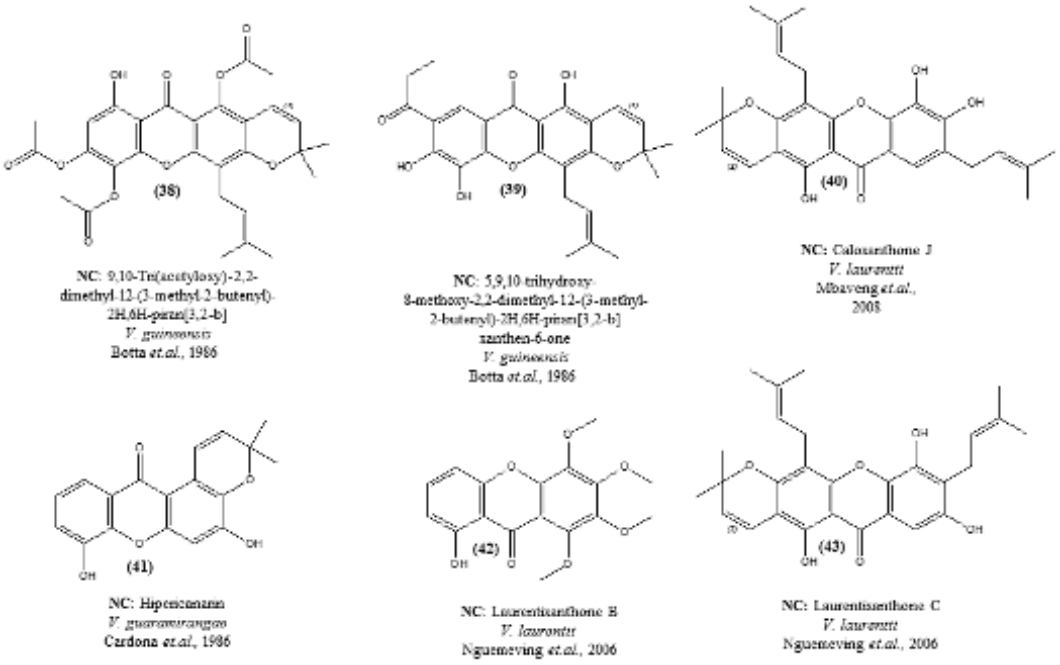


Fig. 7.10 Xanthones isolated from different species of *Vismia* genus.

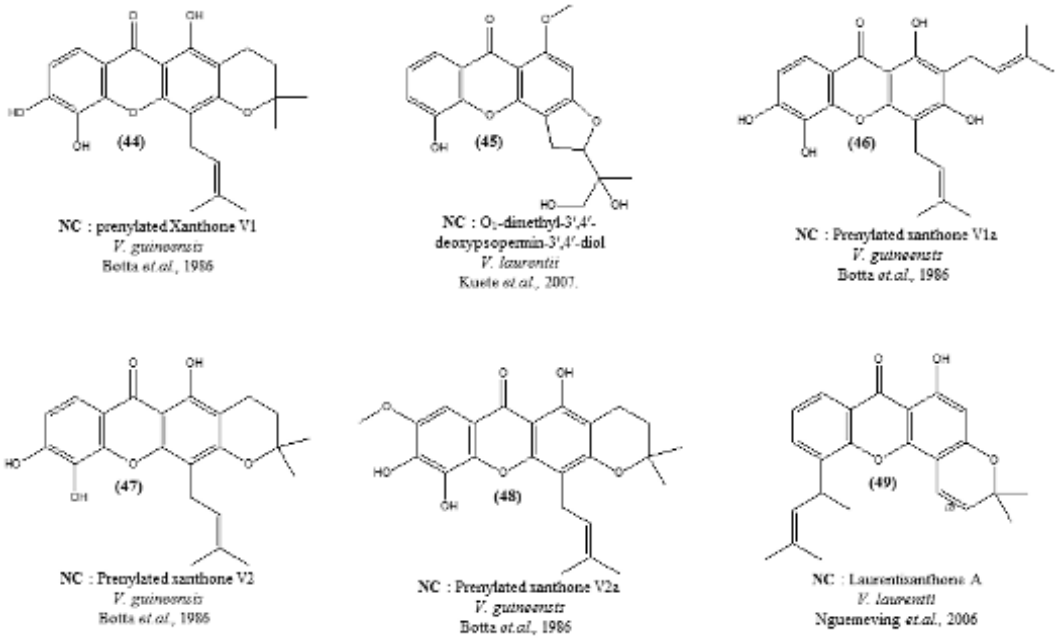


Fig. 7.11 Xanthones isolated from different species of *Vismia* genus.

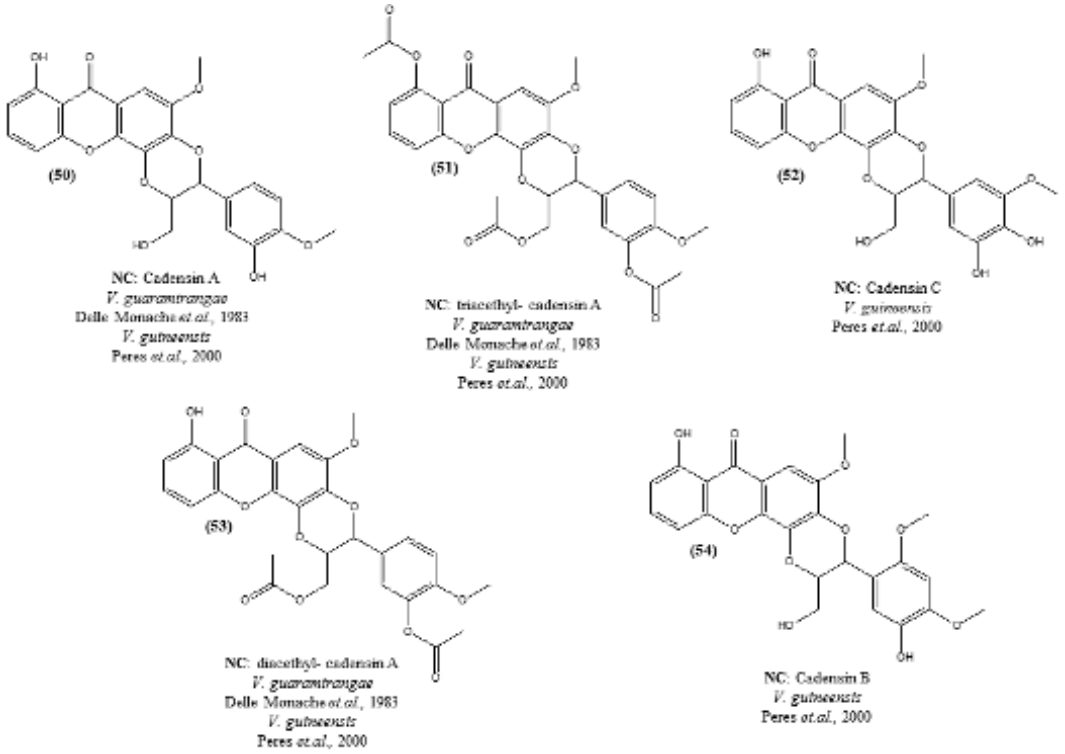


Fig. 7.12 Cadensins isolated from different species of *Vismia* genus.

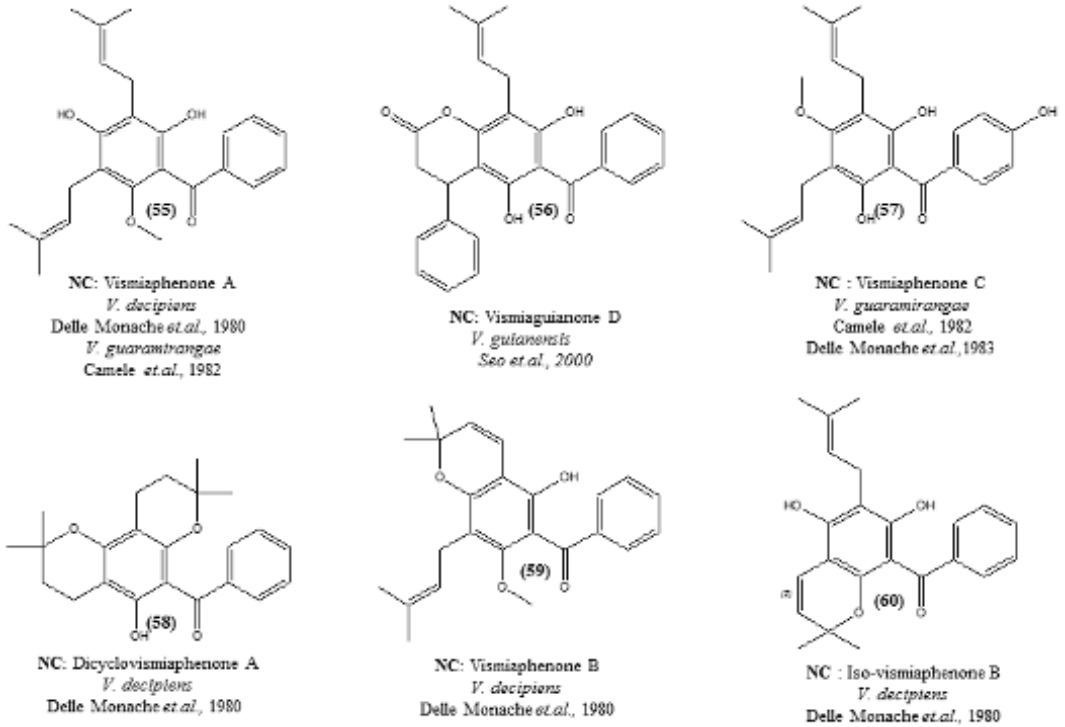


Fig. 7.13 Benzophenones isolated from different species of *Vismia* genus.

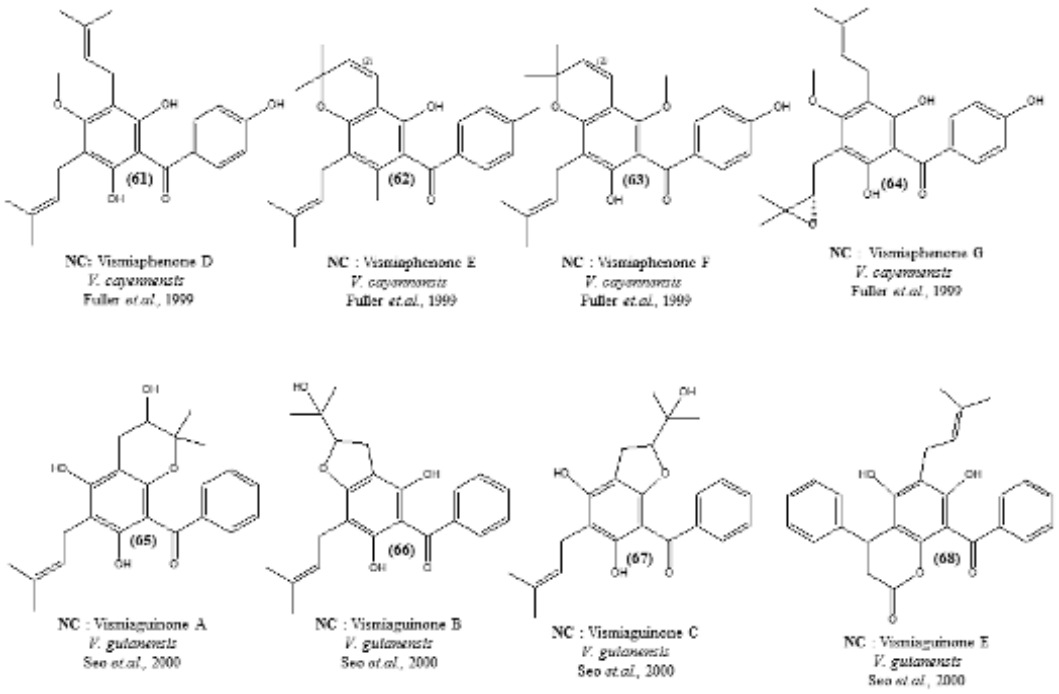


Fig. 7.14 Benzophenones isolated from different species of *Vismia* genus.

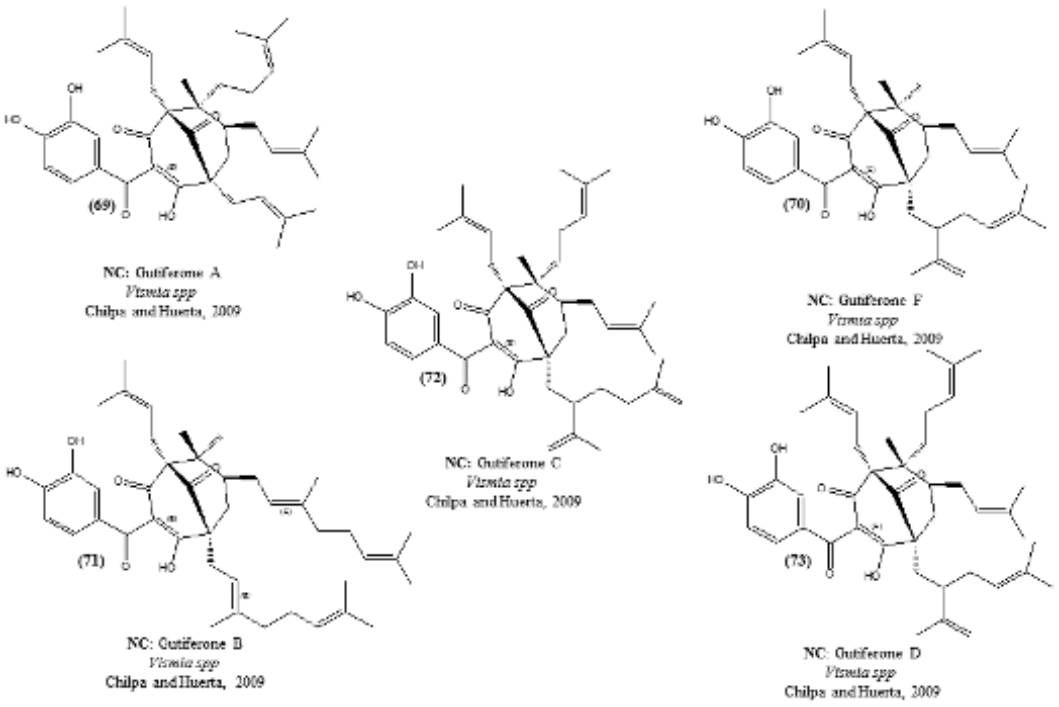


Fig. 7.15 Benzophenones isolated from different species of *Vismia* genus.

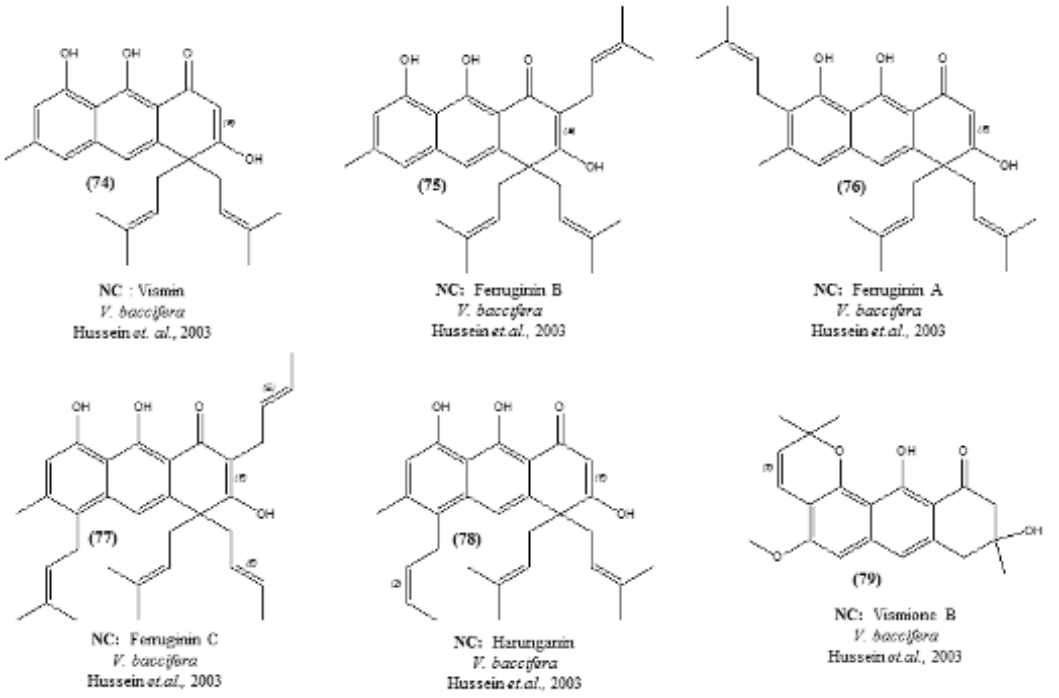


Fig. 7.16 Prenylated anthranoids isolated from different species of *Vismia* genus.

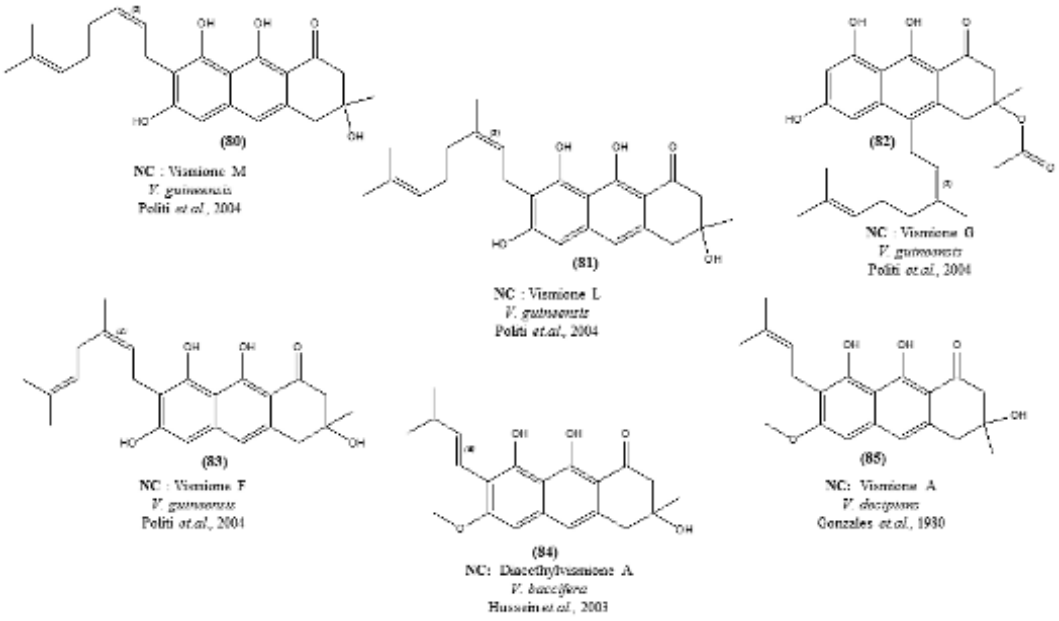


Fig. 7.17 Prenylated anthranoids isolated from different species of *Vismia* genus.

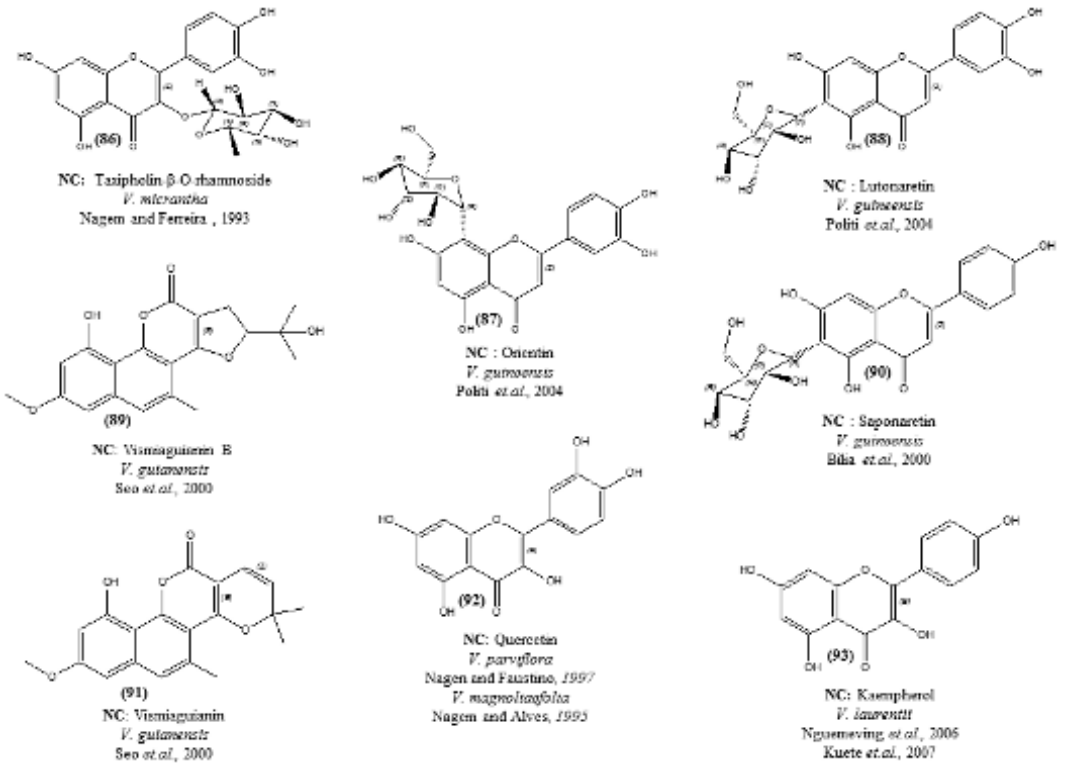


Fig. 7.18 Flavonoids and coumarins isolated from different species of *Vismia* genus.

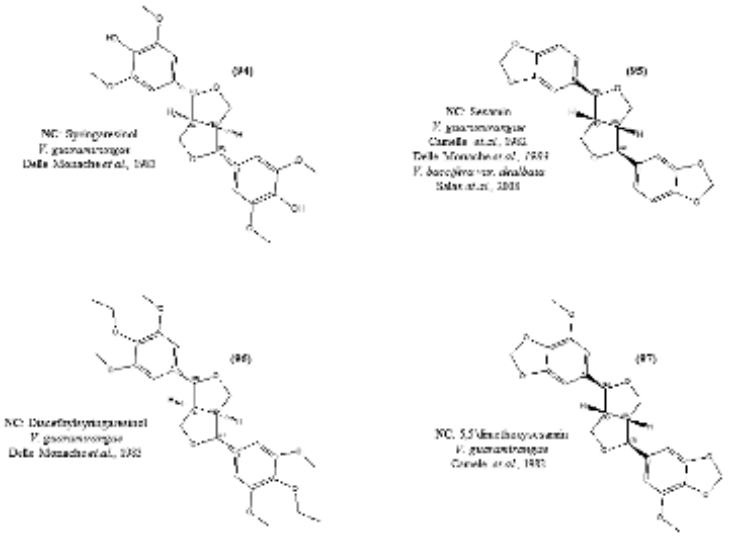


Fig. 7.19 Lignans isolated from different species of *Vismia* genus.

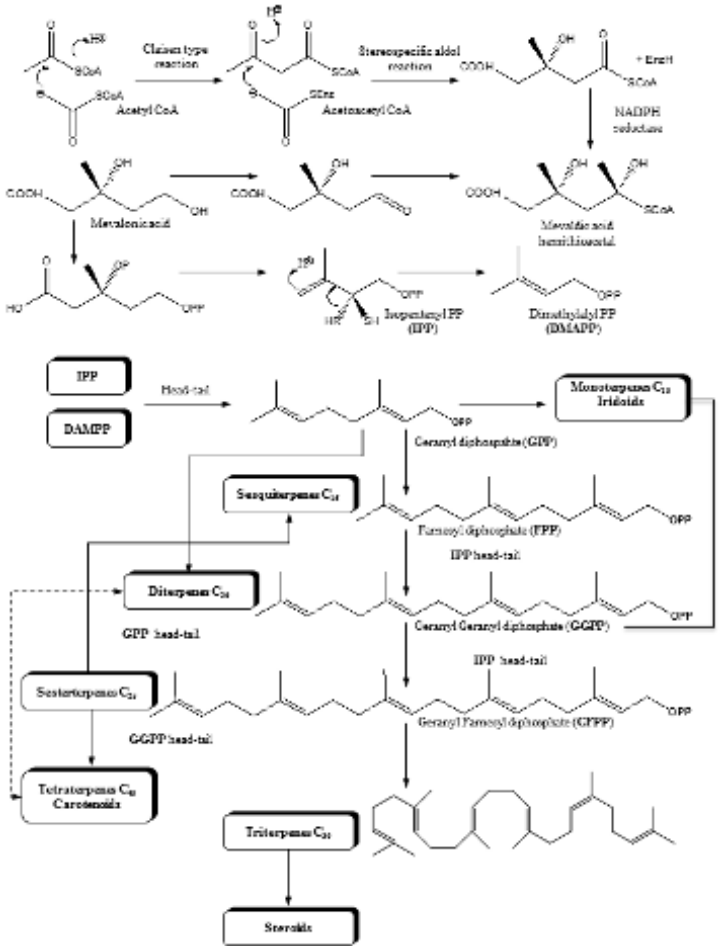


Fig. 7.20 Mevalonate pathway (Dewick 2002, Marcano and Hasegawa 2002).

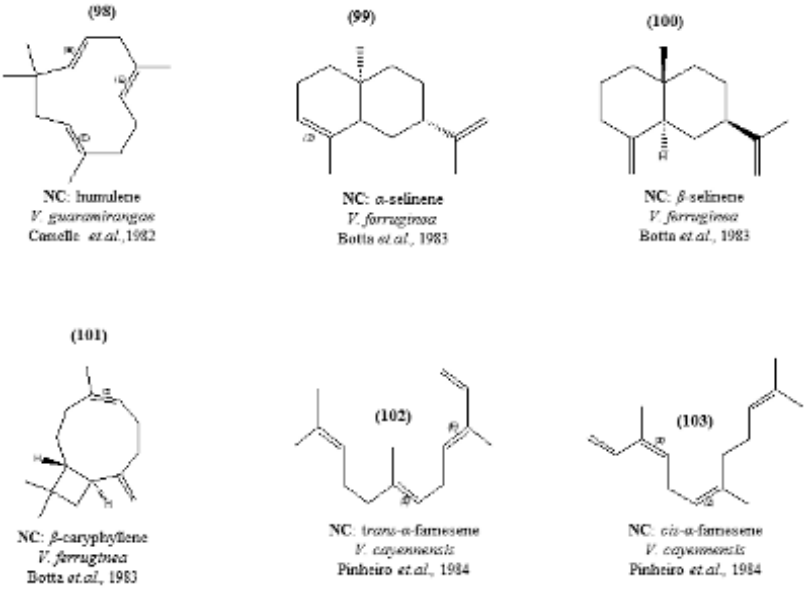


Fig. 7.21 Sesquiterpenes isolated from several *Vismia* especies.

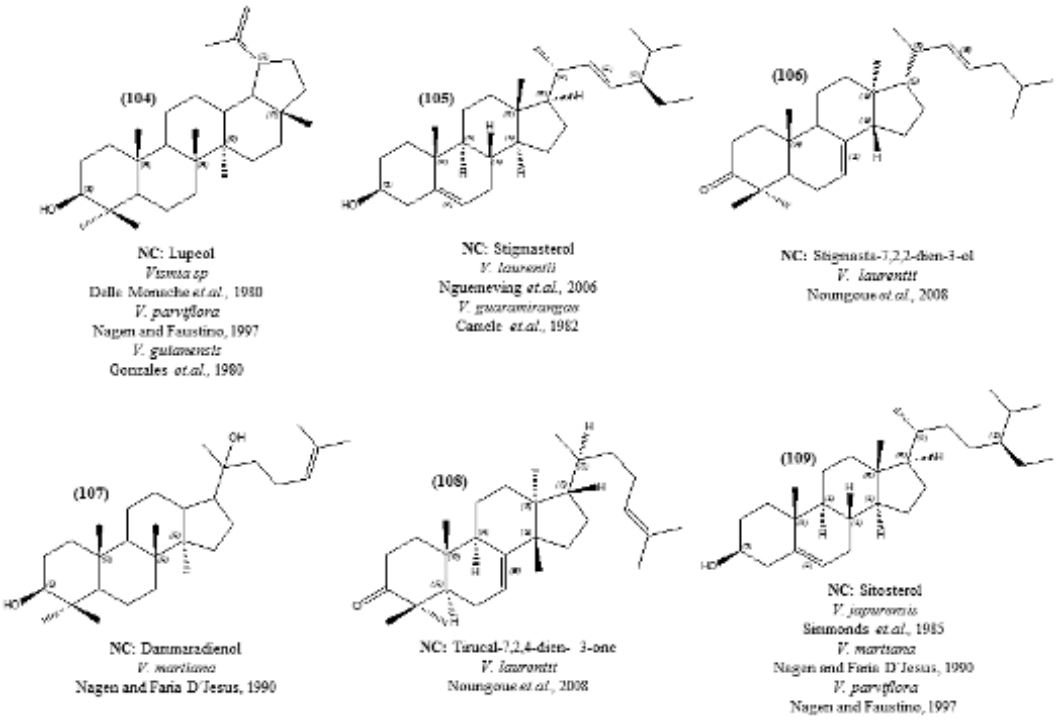


Fig. 7.22 Triterpenes isolated from several *Vismia* especies.

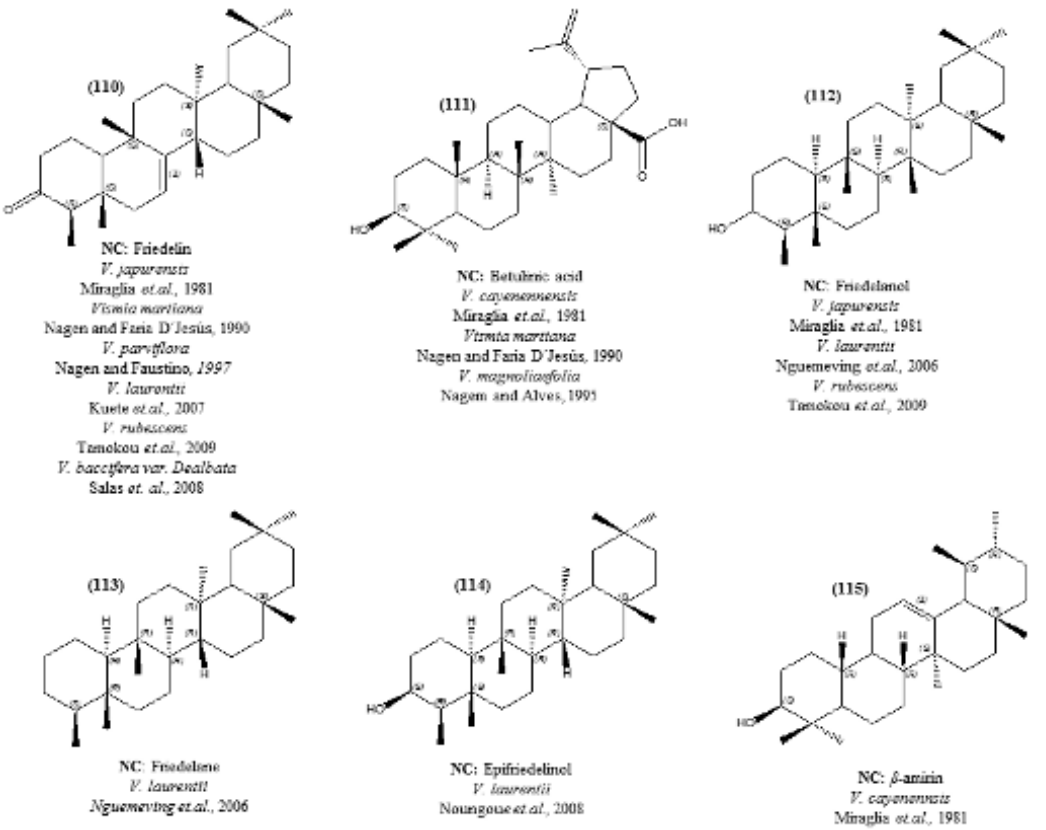


Fig. 7.23 Triterpenes isolated from several *Vismia* especies.

organs, cells, enzymes and receptors). Different parameters are evaluated, such as; growth inhibition, reproduction, morphological, physiological and histological changes (Rahman et al. 2001). Tables 7.2 to 7.10 describe in detail a variety of biological studies, carried out in several species of *Vismia* genus, reported in the last 20 years.

Comparative analysis of secondary metabolites and biological activities studied on species of *Vismia* genus

The interest for the study of *Vismia* genus dates back approximately 25 years, when the researcher, Delle Monache along with his investigation group reported the first study on *Vismia baccifera* var. *ferruginea.* In this investigation, several metabolites were isolated, such as prenylated anthranoids like harunganin (**78**), ferruginin A (**76**) and B (**75**) (Delle Monache et al. 1979). The same research group reported five years later, on an anthraquinone derivative called γ-hydroxyanthrone A_3 (**21**), isolated and purified from *Vismia guaramirangae* (Delle Monache et al. 1983).

To date, at least 161 secondary metabolites have been reported for different species of *Vismia* genus, exhibiting a variety of structures. It is important to state that the majority of compounds have been described for *Vismia guineensis*, *V. guaramirangae* and *V. laurentii.* Figure 7.24 shows the diversity of components isolated from different *Vismia* species, standing out of 42 anthraquinones (Hussain et al. 2012, Vizcaya et al. 2012), vismiaquinone A (**1**), B (**2**) and C (**5**) (Goncalves and Mors 1981, Miraglia et al. 1981, Nagem and Faria D' Jesús 1990, Nagen and Faustino 1997, Hussein et al. 2003, Nguemeving et al. 2006, Salas et al. 2008, Vizcaya et al. 2012) being the most commonly isolated, as well as laurentiquinone A (**3**), B (**4**) and C (**6**) (Nguemeving et al. 2006, Noungoue et al. 2008).

Furthermore, a significant number of xanthones have also been isolated, such as 1,4,8-trihydroxyxanthone (**27**), euxanthone (**28**), 6-deoxyjacareubin (**29**), 1-hydroxy-3,6-dimethoxy-8-methyl-9h-xanthen-9-one (**33**), among others (Dos Santos et al. 2000, Nguemeving et al. 2006, Tamokou et al. 2009, Buitrago et al. 2010, Vizcaya et al. 2012). Compounds found in minor proportions like anthrones, bianthrones (Delle Monache et al. 1979, Gonzales et al. 1980, Delle Monache et al. 1983, Nagem and Faria D'Jesús 1990, Nagem and Alves 1995, Politi et al. 2004, Nguemeving et al. 2006, Hussain et al. 2012, Vizcaya et al. 2012), prenylated anthranoids (Gonzales et al. 1980, Hussein et al. 2003, Mbwambo et al. 2004, Politi et al. 2004) and benzophenones (Delle Monache et al. 1980, Camelle et al. 1982, Delle Monache et al. 1983, Fuller et al. 1999, Seo et al. 2000, Chilpa and Huerta 2009), have also been isolated.

Terpenes are other types of components present in *Vismia* species but in low concentrations, such as triterpenes like, stigmasterol (**105**), lupeol (**104**), fridelin (**110**) and fridelane (**113**), the most commonly isolated (Gonzales et al. 1980, Miraglia et al. 1981, Camelle et al. 1982, Nagem and Faria D' Jesús 1990, Nagen and Faustino 1997, Nguemeving et al. 2006, Kuete et al. 2007, Salas et al. 2008, Tamokou et al. 2009, Vizcaya et al. 2012), similarly, sesquiterpenes such as humulene (**98**), *α*-selinene (**99**), *β*-selinene (**100**) and *β*-caryophyllene (**101**) have been identified in essential oils studied from several *Vismia* species (Camelle et al. 1982, Botta et al. 1983, Buitrago et al. 2009, Rojas et al. 2011a, Rojas et al. 2011b, Vizcaya et al. 2014, Buitrago et al. 2015).

Other compounds isolated from this genus are flavonois; kaempherol (**93**), quercetin (**92**), orientin (**87**), lutonaretin (**88**); coumarins such as vismiaguianin (**91**) and B (**89**) reported for *Vismia guiannensis* (Camelle et al. 1982, Botta et al. 1983, Nagem and Alves 1995, Nagen and Faustino 1997, Seo et al. 2000, Politi et al. 2004, Nguemeving et al. 2006, Kuete et al. 2007) and lignans like sesamin (**95**) commonly found in different *Vismia* species (Salas et al. 2008, Vizcaya et al. 2012).

There are around 31 investigations published in different areas regarding biological activities, with antimicrobial and antioxidant being the most commonly reported mainly for *Vismia guianensis*, *V. laurentii* and *V. baccifera* var. *dealbata*. Figures 7.25 and 7.26, displays biological activities carried out during the last 20 years in extracts, essential oils and pure isolated compounds obtained from different *Vismia* species.

Concerning antimicrobial activity, an anthraquinone named fiscion isolated from *Vismia rubescens* showed activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida parapsilosis* and *Cryptococcus neoformans* with **MIC** values of 7 μg/mL (Tamokou et al. 2009). Similarly, compounds like 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (**7**) and 6-deoxyisojacareubin (**29**) isolated from *Vismia*

Table 7.2 Antimicrobial activity reported for essential oils obtained from *Vismia* species.

Botanical name	Part of the plant	Location	Compounds	Activity	References
Vismia macrophylla	Essential oil from leaves and fruits	Venezuela	**Leaves**: *γ*-bisabolene (44.4%) *β*-bisabolol (14.9%) **Fruits**: *γ*-bisabolene (22.3%) germacrene-*D* (12.1%) *δ*-cadinene (10.7%)	**Leaves:** *S. aureus* (150 µL/mL) *E. faecalis* (250 µL/mL) *E. coli* (740 µL/mL) **Fruits:** *S. aureus* (100 µL/mL) *E. faecalis* (500 µL/mL) *C. albicans* (600 µL/mL) *C. krusei* (600 µL/mL)	Buitrago et al. 2015
Vismia baccifera	Essential oil from bark	Venezuela	caryophyllene oxyde (31.4%), *β*-caryophyllene (26.4%), *α*-zingibirene (12.6%)	*C. tropicalis* (1000 µg/mL) *C. parapsilosis* (1000 µg/mL) *C. neoformans* (1000 µg/mL) *C. krusei* (1.6 µg/mL) *C. glabrata* (200 µg/mL)	Vizcaya et al. 2014
Vismia guianensis	Essential oil from fruits	Africa	*β*-caryophyllene (25.8%), *α*-copaene (13.1%) *δ*-cadinene (11.6%)	*B. cereus* (10 µL/mL) *S. aureus* (10 µL/mL) *S. epidermidis* (10 µL/mL) *S. lentus* (10 µL/mL) *V. alginolyticus* (100 µL/mL)	Silvestre et al. 2012
Vismia guianensis	Leaves	Venezuela	Methanolic extract (**M**) Dichlorometane/ethyl acetate extract (**DA**)	*E. coli* **M, DA** (1 mg/mL) *K. pneumoniae* **DA** (1mg/mL)	Nuñez et al. 2013

Table 7.3 Antimicrobial activity reported for isolated compounds from *Vismia* species.

Botanical name	Part of the plant	Location	Compounds	Activity	References
Vismia laurentii	Leaves	Camerun	laurentixanthone C (**43**) vismiaquinone A (**2**) bisvismiaquinone (**24**) dammaradienol (**107**)	**43**: *C. fusca* **MIC**: 5 mg/mL *B. megaterium* **MIC**: 1 mg/mL.	Tala et al. 2007
Vismia laurentii	Seeds	Camerun	laurenquinone A (**3**) laurenquinone B (**4**) xanthone V1 (**46**) physcion (**8**) 3-geranyloxyemodin anthrone (**22**), friedelin (**110**)	**3**, **8**, **22** *C. fusca* **MIC**: 1 mg/mL **46**: *B. megaterium* **MIC**: 1 mg/mL	Wabo et al. 2007
Vismia baccifera subsp. *dealbata*	Aerial parts	Venezuela	sesamin (**95**) friedelin (**110**) vismiaquinone A (**2**)	*E. faecalis* **95** (2 mg/mL) **110** (4 mg/mL) **2** (4 mg/mL) *P. aeruginosa* **95** (4 mg/mL) **110** (5 mg/mL)	Salas et al. 2007a

Table 7.4 Antiinflammatory and antinoniceptive activities reported for extracts obtained from *Vismia* species.

Botanical name	Part of the plant	Location	Extracts	Activity	References
Vismia guianensis	Leaves	Brazil	Hexane Extract	Concentrations of 100, 200 and 400 mg/Kg decreased 8 times the induced abdominal contortions (**IAC**) by intraperitoneal injection of acetic acid in mice in comparison to reference solution, Indomethacin® (15 mg/kg)	Ferreira et al. 2015
				Biphasic analgesic activity model (noniceptive and inflammatory). Concentrations of 100, 200 and 400 mg/kg, significantly diminished both phases of pain when compared to the control group	
Vismia baccifera subsp. *dealbata*	Leaves	Venezuela	Aqueous Extract	Acute toxicity (**AT**) was measured through the raised paw sign in mice. Extract and control solution were administrated by intraperitoneal injection. Concentration of 420 mg/Kg lacked of any lethal or abnormal behavior on experimental animals	Salas et al. 2007b
				Pain was induced by applying thermal stimuli at mice tails. Analgesic activity was observed after 30 minutes of extract administration, at concentration of 210 mg/kg and it was comparable to the control, acetylsalicylic acid	

laurentii, caused growth inhibition of *Bacillus subtilis* and *Bacillus stearothermophilus* at concentration of 1.22 µg/mL (Kuete et al. 2007).

Essential oils obtained from several *Vismia* species like *Vismia macrophylla*, *V. baccifera* var. *dealbata* and *V. guianensis,* composed mainly by a mixture of volatile type components such as monoterpenes and

Table 7.5 Cardiotonic activity reported for extracts obtained from *Vismia* species.

Botanical name	Part of the Plant	Location	Extracts	Activity	References
Vismia reichardtiana	Leaves	Brazil	Ethanolic Extract	**Hypotensive effect on adult mice** (blood pressure 93.1 ± 3.8 mmHg) Intravenous administration of extract at concentrations between 0.5 to 30 mg/Kg	Gomes et al. 2009
				Reference drug: Acetylcholine (**Ach**) Noradrenaline (**Nor**) 0.5 µg/kg Atropine (**Atr**) 1 mg/kg	
				Extract showed hypotensive effect (13.8 ± 3.7 mmHg), at concentration of 20 mg/kg, compared to **Nor** (13.2 ± 3.3 mmHg)	

Table 7.6 Antioxidant activity reported for extracts and isolated compounds obtained from *Vismia* species.

Botanical name	Part of the plant	Location	Extracts/compounds	Activity	References
Vismia baccifera	Leaves	Venezuela	Methanol extracts **VB** (*V. baccifera*) **VM** (*V. macrophylla*)	**DPPH:** **VB** (**IC_{50}**: 5.50 µg mL^{-1}) **Folin-Ciocalteu:** **VB** 306.21 mg Eq AG/g Ext **Flavonoids content** **VB** 267.07 mg Eq Que/g Ext	Buitrago et al. 2016
Vismia macrophylla				**DPPH:** **VM** (**IC_{50}**: 5.87 µg mL^{-1}) **Folin-Ciocalteu:** **VM** 391.28 mg Eq AG/g Ext **Flavonoids content** **VM** 185.90 mg Eq Que/g Ext	
Vismia guianensis	Fruits	Brazil	Essential Oil	Antioxidant activity by emulsion discoloration of β-carotene (yellow color). Reference: Trolox (**Trx**). At concentration of 100 µL, the essential oil showed 70% of inhibition comparing to **Trx** (60%)	Silvestre et al. 2012
Vismia rubescens	Stems	Cameroon	friedelin (**110**) friedelanol (**112**) lupeol (**104**) 1,7-dihydroxyxanthone (**4**) 1,4,8-trihydroxyxanthone (**27**) 1,2,8-trihydroxyxanthone (**32**) physcion (**8**)	**Extract** **IC_{50}**: 2.18 µg mL^{-1} **Compounds:** **27** (**IC_{50}**: 1.96 µg mL^{-1}) **32** (**IC_{50}**: 1.73 µg mL^{-1}) Reference drug Ascorbic acid (**IC_{50}**: 1.86 µg/mL)	Tala et al. 2011

sesquiterpenes, showed a wide range of activity against several bacteria strains such as *Staphylococcus aureus* at **MIC** values between 10 to 100 µL/mL (Silvestre et al. 2012, Buitrago et al. 2015); *Escherichia*

Table 7.7 Antioxidant activity reported for extracts and isolated compounds obtained from *Vismia* species.

Botanical name	Part of the plant	Location	Extracts/compounds	Activity	References
Vismia baccifera subsp. *ferruginea*	Fruits	Colombia	Petroleum ether Ethyl acetate Methanol	**Total Phenols: Petroleum ether** 78.33 mg AG/g Ext **Ethyl acetate** 350.56 mg AG/g Ext **Methanol** 177.22 mg AG/g Ext **DPPH**: IC_{50}: 4.46 μg mL^{-1} **ABTS**: IC_{50}: 4.16 μg mL^{-1}	Álvarez et al. 2009
Vismia guianensis				**Total Phenols: Ethyl acetate** 356.67 mg AG/g Ext **Methanol** 186.67 mg AG/g Ext **Petroleum ether** 205 mg AG/g Ext **DPPH**: IC_{50}: 3.72 μg mL^{-1} **ABTS**: IC_{50}: 5.86 μg mL^{-1}	
Vismia laurentii	Seeds	Cameroon	laurenquinone A (**3**) laurenquinone B (**4**) xanthone V1 (**46**) laurentixanthone C (**43**) bivismiaquinone (**24**) vismiaquinone A (**2**) vismiaquinone B (**1**)	**Extract:** (**IC_{50}**: 2.57 μg mL^{-1}) **Compounds** **3** (**IC_{50}**: 4.78 μg mL^{-1}) **46** (**IC_{50}**: 2.09 μg mL^{-1}) **1** (**IC_{50}**: 2.09 μg mL^{-1})	Tala et al. 2011

Table 7.8 Parasiticide activity reported for extracts and isolated compounds obtained from *Vismia* species.

Botanical name	Part of the plant	Location	Extracts/compounds	Activity	References
Vismia laurentii	Bark	Camerun	Hexane extract (HE) Ethyl acetate extract (EAE) Compounds: Tirucalla-7,2,4-dien-3-one (**108**), 3-geranyloxyemodin (**22**), vismiaquinone A (**2**), vismiaquinone B (**1**), bivismiaquinone (**24**), epyfriedelinol (**112**), betulinic acid (**111**) stigmasta-7,22-dien-3-ol (**106**)	Activity against *Plasmodium falciparum* Reference drug Chloroquine phosphate 1 μM **HE:** IC_{50} 4.6 μg/mL **EAE:** IC_{50} 4.6 μg/mL **108**: IC_{50} 1.18 μg/mL **2**: IC_{50} 1.42 μg/mL	Noungoue et al. 2009
Vismia baccifera subsp. *ferruginea*	Leaves	Colombia	Ethanol Extract	*In vitro* activity against promastigotes and amastigotes of *T. cruzi*, *L. amazonensis*, *L. donovani* and *L. brazilensis* **IC_{50}:** 42.9 to 71.7 mg/mL	Gallego et al. 2006

coli (**MIC**: 740 μL/mL) and *Enterococcus faecalis* (**MIC**: 500 μL/mL), as well as yeasts like *Candida albicans* (**MIC**: 600 μL/mL), *C. krusei* (**MIC**: 600 μL/mL) and *C. glabrata* (**MIC**: 200 μg/mL).

On the other hand, the search for natural sources with antioxidant activity has caught the attention of scientists since oxidative stress in cells might promote several pathologies such as cancer, diabetes mellitus, hepatic ailments, inflammation, premature aging, among others (Sarabjot and Poonam 2014).

Table 7.9 Anti-VIH activity reported for extracts and isolated compounds from *Vismia* species.

Botanical name	Part of the plant	Location	Extracts/ compounds	Activity	References
Vismia mexicana	Leaves	Mexico	Dichloromethane/ Methanol (1:1)	**VIH Reverse transcriptase** Positive control: Nevirapina® Enzyme inhibition **Vm** $\mathbf{IC_{50}}$ 36.17 µg mL^{-1}	Gómez-Cansino et al. 2015
Vismia baccifera				**Vb** $\mathbf{IC_{50}}$ 31.75 µg mL^{-1}	
Vismia mexicana	Leaves	Mexico	Dichloromethane/ Metanol (1:1)	**VIH Reverse transcriptase** Inhibition of viral replication At concentration of 50 µg/mL ***Vm***: $\mathbf{IC_{50}}$ 72.9%	Huerta-Reyes et al. 2004
Vismia camparaguey				***Vc,*** $\mathbf{IC_{50}}$ 70.8%	

Table 7.10 Anticancer activitity reported for extracts and isolated compounds from *Vismia* species.

Botanical name	Part of the plant	Location	Extracts/compounds	Activity	References
Vismia baccifera	Leaves	Colombia	Aqueous Extract	Anticancer activity on human hepatocellular carcinoma **HepG2**, **PLC/PRF/5** **SK-HEP-1** **Vb** (19 µg/mL) showed selective growth inhibition of **HepG2**	Lizcano et al. 2015
Vismia baccifera subsp. *dealbata*	Leaves	Venezuela	Sesamin (**95**)	Anticancer activity on **SK-OV-3** **PC3** **NCI-H292** At concentration of $\mathbf{IC_{50}}$: 1000 mg/mL, sesamin showed growth inhibition of **NCI-H292**	Salas et al. 2008
Vismia baccifera	Leaves	Venezuela	Different polarity fractions obtained from methanolic extracts: Hexane (**Hex**) Dichloromethane (**DCl**) Ethyl Acetate (**EthAc**) Buthanol (**But**) Water (**Wt**) Ethyl Acetate/Buthanol (**EthAc/But**)	Cytotoxic activity ($\mathbf{IC_{50}}$) **Vb**: **PC3**: 2.92 µg/mL **DCl** **HeLa**: 28.81 µg/mL **Hex** **Vm**: **MCF-7**: 36.25 µg/mL **Wt** **SKBr3**: 12.14 µg/mL **Wt** **PC3**: 10.91 µg/mL **Wt** **HeLa**: 6.09 µg/mL **Hex**	Rojas et al. 2017
Vismia macrophylla					

Plant extracts have revealed to be a major source of chemical compounds with high antioxidant activity and several assays; Folin-Ciocalteu, **DPPH•** and **ABTS•+**, among others, have been carried out in extracts of *Vismia rubescens, V. laurentii, V. baccifera* ssp. *ferruginea* and *V. guianensis* and showed free radical scavenger activity at concentrations between 4.46 to 2.18 µg/mL (Álvarez et al. 2009, Tala et al. 2011, Silvestre et al. 2012).

Antioxidant activity has also been demonstrated in methanolic extracts of *Vismia baccifera* and *V. macrophylla,* at $\mathbf{IC_{50}}$ values of 5.87 and 5.35 µg/mL, respectively (Buitrago et al. 2016). Isolated

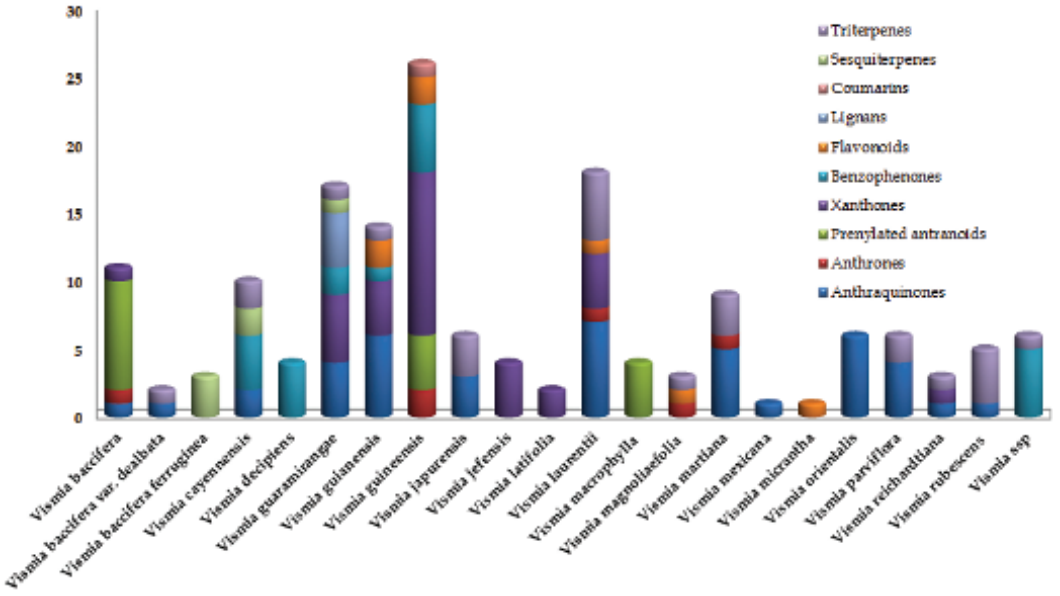


Fig. 7.24 Secondary metabolites isolated from different *Vismia* species

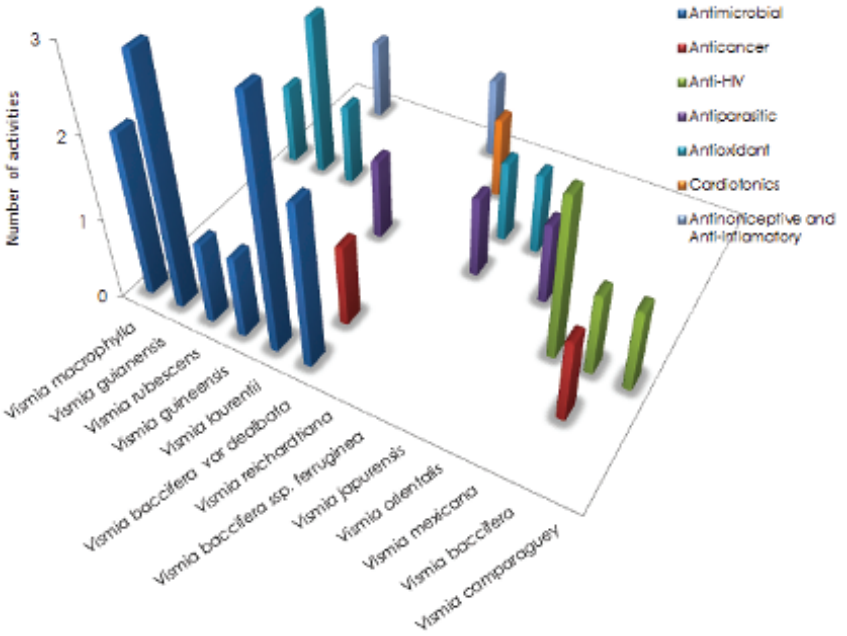


Fig. 7.25 Biological activity assays reported for *Vismia* species in the last 20 years.

compounds; 1,4,8-trihydroxyxanthone (**27**) and 1,2,8-trihydroxyxanthone (**32**), from *Vismia rubescens,* also showed free radical scavenger at $\mathbf{IC}_{50}$ of 1.96 µg/mL and 1.73 µg/mL, respectively (Tala et al. 2011).

Cardiotonic, hypotensive, noniceptive, analgesic and anti-VIH activitites has also been published for *Vismia* genus. Regarding cardiotonic and hypontesive activitites, intravenous administration of *Vismia reichardtiana* leaves extract at concentrations between 0.5 to 30 mg/Kg caused hypotensive effect on adult mice comparable to the reference drugs noradrenaline® and acetylcholine® (Gomes et al. 2009). Analgesic activity of hexanic extract of *Vismia guianensis* leaves was assayed *in vivo* in experimental animals; results showed a decrease in pain threshold after oral administration of such extract at concentrations between

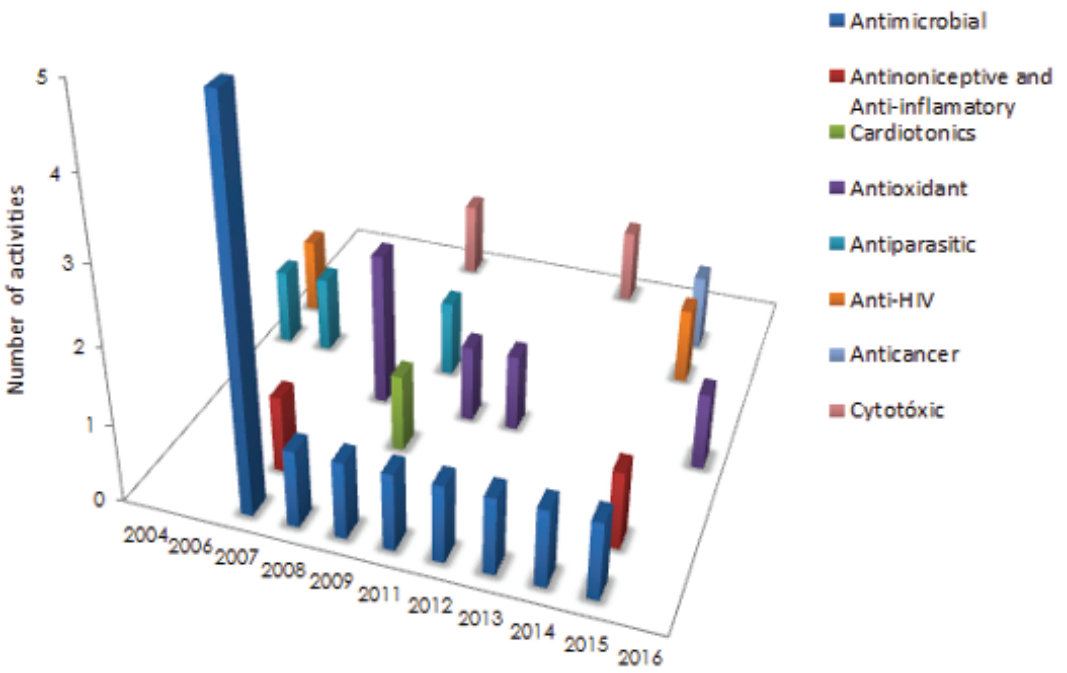


Fig. 7.26 Biological activity assays reported for different *Vismia* species.

100 to 400 mg/kg of animal weight (Ferreira et al. 2015). *V. mexicana* and *V. baccifera* were studied for VIH reverse transcriptase activity, revealing inhibition of viral replication at concentration of 35 µg/mL (Gómez-Cansino et al. 2015).

Conclusions

According to investigations published for *Vismia* genus, it might represent a source of natural compounds with a wide range of biological activities. Nowdays, there is a huge interest for the search of new active molecules that might be used to develop pharmaceutical forms for the treatment of diverse pathologies. However, it is very important to bear in mind that natural products come from plants and excessive, disproportionate use of these may cause the extinction of vulnerable species, thus, it is necessary to promote the protection and conservation of natural resources focused in sustainable conditions.

References

Álvarez, E., Jímenez, J.G., Posada, M.A., Rojano, B., Gil, J.H., García, C.M. and Durango, D.L. 2008. Actividad antioxidante y contenido fenólico de los extractos provenientes de las bayas de dos especies del género *Vismia* (Guttiferae). VITAE, Revista de La Facultad de Química Farmacéutica 15(1): 165–172.

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161(2): 105–121.

Bilia, A.R., Yusuf, A.W., Braca, A., Keita, A. and Morelli, I. 2000. New prenylated anthraquinones and xanthones from *Vismia guineensis*. J. Nat. Prod. 63: 16–21.

Botta, B., Delle Monache, G., Delle Monache, F., Bettolo, M. and Oguakwa, U. 1983. 3-geranyloxy-6-methyl-1,8-dihydroxy anthraquinone and Vismiones C, D and E from Psorospermum febrifugum. Phytochem. 22: 539–542.

Botta, B., Delle Monache, F., Delle Monache, G., Bettolo, M. and Menichini, F. 1986. Vismione H and prenilated Xanthones from *Vismia guineensis*. Phytochem. 25: 1217–1219.

Buitrago, A., Rojas, L.B., Rojas, J., Buitrago, D., Usubillaga, A. and Morales, A. 2009. Comparative study of the chemical composition of the essential oil of *Vismia baccifera* var. *dealbata* (Guttiferae) collected in two different locations in Merida-Venezuela. J. Essent. Oil Bear Pl. 12(6): 651–655.

Buitrago, A., Rojas, J., Cote, V., Bruno-Colmenárez, J. and Díaz de Delgado, G. 2010. NMR elucidation and crystal structure analysis of 1-hydroxy-3,6-dimethoxy-8-methyl-9h-xanthen-9-one (lichexanthone) isolated from *Vismia baccifera* (Guttiferae). BLACPMA 9(6): 470–474.

Buitrago, A., Rojas, J., Rojas, L., Velasco, J., Morales, A., Peñaloza, Y. and Díaz, C. 2015. Essential oil composition and antimicrobial activity of *Vismia macrophylla* leaves and fruits collected in Táchira-Venezuela. Nat. Prod. Commun. 10(2): 375–377.

Buitrago, A., Rojas, J., Rojas, L. and Peñaloza, Y. 2016. *In Vitro* antioxidant activity and qualitative phytochemical analysis of two *Vismia* (Hypericaceae) species collected in Los Andes, Venezuela. Rev. Biol. Trop. 6(4): 1431–1439.

Camelle, G., Delle Monache, F., Delle Monache, G., Martini Bettolo, B. and Alves de Lima, R. 1982. 2-isoprenylemodin and 5,5´-dimethoxysesamin from Vismia guaramirangae. Phytochem. 21: 417–419.

Cardona, L., Fernández, I., Pedro, J., Seoane, E. and Vidal, R. 1986. Additional new xanthones and xanthonolignoids from Hypericum canariensis. J. Nat. Prod. 49: 95–100.

Chilpa, R. and Reyes, M. 2009. Compuestos naturales de plantas de la familia Clusiaceae: Inhibidores del virus de inmunodeficienciaia humana tipo 1. Interciencia 34: 385–392.

Crocketta, S.L. and Robson, N.K. 2011. Taxonomy and chemotaxonomy of the genus Hypericum. Med. Aromat Plant Sci. Biotechnol. 5(1): 1–13.

Delle Monache, F., Marquina, M., Ferrari, F. and Martini-Bettolo, G. 1979. Ferruginin A and B and Ferruanthrone, New triprenylated anthranoids from *Vismia baccifera* var. *ferruginea*. Tetrahedrom. 35: 2143–2149.

Delle Monache, G., Gonzales, J., Delle Monache, F. and Marini, G. 1980. Prenylated benzophenones from *Vismia decipiens*. Phytochem. 19: 2025–2028.

Delle Monache, F., Marquina Mac-Quhae, M., Delle Monache, G., Marini, G. and Alves de Lima, R. 1983. Xanthones, xantholignoids and others constituents of the roots of *Vismia guaramirangae*. Phytochem. 22: 227–232.

Dewick, P.M. 2002. The biosynthesis of C_5–C_{25} terpenoid compounds. Nat. Prod. Rep. 19: 181–222.

Doriguetto, A., Santos, M., Ellena, J. and Nagen, T. 2001. 6-deoxyjacareubin. Acta Crystallogr C 57: 109–1079.

Dos Santos, M., Nagem, T., Da Silva, M. and Silva, L. 2000. Xanthones from *Vismia latifolia*. J. Braz. Chem. Soc. 11: 537–539.

Epifano, F., Genovese, S., Menghini, L. and Curini, M. 2007. Chemistry and pharmacology of oxyprenylated secondary plant metabolites. Phytochem. 68: 939–953.

Fuller, R., Westergaard, C., Collins, J., Cardellina, J. and Boyd, M. 1999. Vismiaphenones D-G from *Vismia cayennensis*. J. Nat. Prod. 62: 57–69.

Ferreira Nobre, V., Marchesine Almeida, D.M., Lucchese, A.M., Lopes Rocha, M., Santos Oliveira, A.T. and Maia Barboza, A.C. 2015. Antinociceptive and anti-inflammatory activity of hexanic extract leaves of *Vismia guianensis* Aubl. in mice. Revista de Ciências Médicas e Biológicas 14(1): 69–73.

Gallego, A., Torres, F., Robledo, S., Vélez, I.D., Carrillo, L., Muñoz, D.L., Quiñones, W., Fonnegra, R., Roldán, J., Valencia, L., Triana, O. and Echeverri, F. 2006. Actividad leishmanicida y tripanocida de *Acacia farnesiana*, *Piper arieianum*, *Piper Subpedale*, *Sphagnum recurvum* y *Vismia baccifera ferruginea*. Actualidades Biológicas 28: 39–49.

Gómez-Cansino, R., Espitia-Pinzón, C.I., Campos-Lara, M.G., Guzmán-Gutiérrez, S.L., Segura-Salinas, E., Echeverría-Valencia, G., Torras-Claveria, L., Cuevas-Figueroa, X.M. and Reyes-Chilpa, R. 2015. Antimycobacterial and HIV-1 Reverse transcriptase activity of Julianaceae and Clusiaceae plant species from Mexico. J. Evid. Based Complementary Altern. Med. 2015: 1–8.

Gomes, M.J.N., Silva, S.N., Ribeiro, R.M., Abreu, I.C., Borges, R.M.O. and Borges, R.A.C. 2009. Screening farmacológico das folhas de *Vismia reichardtiana* (O. Ktze) Ewan-Guttiferae. Revista Cadernos de Pesquisa 16(1): 5–10.

Goncalves, M. and Mors, W. 1981. Vismiaquinone A. δ-isopentenyl substituted anthraquinone from Vismia reirchartiana. Phytochem. 20: 1947–1950.

Gonzales, J., Delle Monache, F., Delle Monache, G. and Marini Bettolo, G.B. 1980. Chemistry of the genus *Vismia*. VII. Vismione A from the leaves of *Vismia guianensis*. Plant Med. 40: 347–350.

Goossens, A., Häkkinen, S., Laakso, I., Seppänen Laakso, T., Biondi, S, De Sutter, V., Lammertyn, F., Nuutila, A.M., Söderlund, H., Zabeau, M., Inze, D. and Oksman-Caldentey, K. 2003. A functional genomics approach toward the understanding of secondary metabolism in plant cells. Proc. Natl. Acad. Sci. 100: 8595–8600.

Hokche, O., Berry, P.E. and Huber, O. 2008. *Nuevo catálogo de la flora vascular de Venezuela*. Caracas, Venezuela: Ediciones Fundación Instituto Botánico de Venezuela "Dr. Tobías Lasser".

Huerta-Reyes, M., Basualdo, M.C., Lozada, L., Jiménez-Estrada, M., Soler, C. and Reyes-Chilpa, R. 2004. HIV-1 inhibition by extracts of Clusiaceae species from México. Chem. Pharm. Bull. 27: 916–920.

Hussain, H., Hussain, J., Al-Harrasi, A., Saleem, M., Green, I.R., van Ree, T. and Ghulam, A. 2012. Chemistry and biology of genus *Vismia*. Pharm. Biol. 50(11): 1448–1462.

Hussein, A., Bozzi, B., Correa, N., Capson, T., Kursar, T., Coley, P., Solis, P. and Gupta, M. 2003. Bioactive constituents from three *Vismia* species. J. Nat. Prod. 66: 858–860.

Kuete, V., Nguemeving, J., Beng, V., Azebaze, A., Etoa, F., Meyer, M., Bodo, B and Nkengfack, A. 2007. Antimicrobial activity of the methanolic extracts and compounds from *Vismia laurentii* De Wild (Guttiferae). J. Ethnopharmacol. 109: 372–379.

Lizcano, L.J., Siles, M., Trepiana, J., Hernández, M.L., Navarro, R.M., Ruiz-Larrea, B. and Ruiz-Sanz, J.I. 2015. *Piper* and *Vismia* species from Colombian Amazonia differentially affect cell proliferation of hepatocarcinoma cells. Nutrients 7: 179–195.

Marcano, D. and Hasegawa, M. 2002. *Fitoquímica orgánica*. Caracas, Venezuela: Ediciones Vicerrectorado Académico UCV.

Mbaveng, A.T., Kuete, V., Nguemeving, J.R., Penlap Beng, V., Nkengfack, A. E., Marion Meyer, J.J., Lall, N. and Krohn, K. 2008. Antimicrobial activity of the extracts and compounds obtained from *Vismia guineensis* (Guttiferae). Asian J. Tradit. Med. 3(6): 211–223.

Mbwambo, Z.H., Apers, S., Moshi, M.J., Kapingu, M.C., Van Miert, S., Claeys, M., Brun, R., Cos, P., Pieters, L. and Vlietinck, A. 2004. Anthranoid compounds with antiprotozoal activity from *Vismia orientalis*. Plant Med. 70(8): 706–710.

Miraglia, M., Mesquita, A., Varejão, M., Gottlieb, O. and Gottlieb, H. 1981. Anthraquinones from *Vismia* species. Phytochem. 20: 2041–2042.

Nagem, J. and Faria de Jesús, T. 1990. Quinoids and other constituents of *Vismia martiana*. Phytochem. 29: 3362–3364.

Nagem, T.J. and Ferreira, M.A. 1993. Constituents of *Vismia micrantha*. Fitoterapia 64: 382–383.

Nagem, T. and Alves, V. 1995. Constituents of *Vismia magnoliaefolia*. Fitoterapia 56(3): 278–280.

Nagen, J. and Faustino, F. 1997. Xanthones and other constituents of *Vismia parviflora*. J. Brazil. Chem. Soc. 8: 505–508.

Nguemeving, J., Azebaze, A., Kuete, V., Carly, N., Beng, P., Meyer, M., Blond, A., Bodo, B. and Nkengfack, A. 2006. Laurentixanthones A and B, antimicrobial xanthones from *Vismia laurentii*. Phytochem. 67: 1341–1346.

Noungoue, D., Antheaume, C., Chaabi, M., Ndjakou, B., Ngoule, S., Lobstein, A. and Tsamo, E. 2008. Anthraquinones from the fruits of *Vismia laurentii*. Phytochem. 69: 1024–1028.

Noungoue, D.T., Chaabi, M., Ngouela, S., Antheaume, C., Boyom, F.F., Gut, J., Rosenthal, P.J., Lobstein, A. and Tsamo, E. 2009. Antimalarial compounds from the stem bark of *Vismia laurentii*. Z. Naturforsch. C. 64c: 210–214.

Nuñez, R., Rojas J., Lucena, M., Roa, A. and Meléndez P. 2013. Evaluación de la actividad antibacteriana y efecto citotóxico de extractos obtenidos de la especie *Vismia guianensis* (Aubl.) Pers. (Hypericaceae). Revista de la Facultad de Farmacia 55(2): 29–34.

Nürk, N.M., Madriñán, S., Carine, M.A., Chase, M.W. and Blattner, F.R. 2013. Molecular phylogenetics and morphological evolution of St. John's wort (Hypericum; Hypericaceae). Mol. Phylogenet. Evol. 66(1): 1–16.

Peres, V. and Nagen, T. 1997. Trioxygenated naturally occurring xanthones. Phytochem. 44: 191–214.

Peres, V., Nagen, T. and Faustino, F. 2000. Tetraoxygentaed naturally occurring xanthones. Phytochem. 55: 683–710.

Pinheiro, R., Mac-Quhae, M., Bettolo, M. and Delle Monache, F. 1984. Prenylated antranoids from *Vismia* species. Phytochem. 23: 1737–1740.

Politi, M., Sanogo, R., Ndjoko, K., Guilet, D., Wolfender, J.L., Hostettmann, K. and Morelli, I. 2004. HPLC-UV/PAD and HPLC-MS (n) analyses of leaf and root extracts of *Vismia guineensis* and isolation and identification of two new bianthrones. Phytochem. Anal. 15: 355–364.

Rahman, A., Choudhary, M.I. and Thomson W.J. 2001. *Bioassay techniques for drug development*. New Jersey, United States: Harwood Academic Publishers.

Rates, S.M.K. 2001. Review. Plants as source of drugs. Toxicon 39: 603–613.

Rojas, J., Buitrago, A., Rojas, L., Morales, A., Lucena, M. and Baldovino, S. 2011a. Essential oil composition and antibacterial activity of *Vismia baccifera* fruits collected from Mérida, Venezuela. Nat. Prod. Commun. 6: 699–700.

Rojas, J., Buitrago, A., Rojas, L. and Morales, M. 2011b. Essential oil composition of *Vismia macrophylla* Leaves (Guttiferae). Nat. Prod. Commun. 6: 85–86.

Rojas, J.C., Buitrago, A.A., Arvelo, F.A., Sojo, F.J. and Suarez, A.I. 2017. Cytotoxic activity of different polarity fractions obtained from methanolic extracts of *Vismia baccifera* and *Vismia macrophylla* (Hypericaceae) collected in Venezuela. JPPRes 5: 320–326.

Ruhfel, B., Bittrich, V., Bove, C., Gustafsson, M., Philbrick, C., Rutishauser, R., Xi, Z. and Davis, C. 2011. Phylogeny of the clusioid clade (Malpighiales): evidence from the plastid and mitochondrial genomes. Am. J. Bot. 98: 306–325.

Salas, F., Velasco, J., Rojas, J. and Morales, A. 2007a. Antibacterial activity of the crude extract and constituents of *Vismia baccifera* var. *dealbata* (Guttiferae) collected in Venezuela. Nat. Prod. Commun. 2(2): 185–188.

Salas, F., Ciangherotti, C., Salazar-Bookaman, M., Rojas, J. and Morales, A. 2007b. Toxicidad aguda y actividad analgésica del extracto acuoso de hojas de *Vismia baccifera* L. var. *dealbata* (Guttiferae) en animales de experimentación. Revista de la Facultad de Farmacia 49(1): 5–9.

Salas, F., Rojas, J., Morales, A. and Ramos-Nino, M. 2008. *In Vitro* cytotoxic activity of sesamin isolated from *Vismia baccifera* var. *dealbata* Triana & Planch (Guttiferae) collected from Venezuela. Nat. Prod. Commun. 3: 1705–1708.

Schultes, R. 1993. Plants in treating senile dementia in the northwest Amazon. J. Ethnopharmacol. 38: 139–135.

Seo, E., Wani, M., Wall, M., Navarro, H., Mulkherjee, R., Farnsworth, N. and Kinghorn, D. 2000. New bioactive aromatic compounds from *Vismia guianensis*. Phytochem. 55: 35–42.

Silvestre, R.G., de Moraes, M.M., Lins, A.C.S., Ralph, M.T., Lima-Filho, J.V., Camara, C.A. and Silva, T.M.S. 2012. Chemical composition, antibacterial and antioxidant activities of the essential oil from *Vismia guianensis* fruits. Afr. J. Biotechnol. 11(41): 9888–9893.

Shilpa, K., Varun, K. and Lakshmi, B.S. 2010. An alternate method of natural drug production: Eliciting secondary metabolite production using plant cell culture. Journal of Plant Sciences 5: 222–247.

Simmonds, M., Blaney, W., Delle Monache, F., Marquina, M. and Marini Bettolo, G. 1985. Insect antifeedant propiertis of anthanoids from the genus *Vismia*. J. Chem. Ecol. 11: 1593–1599.

Stevens, P.F. 2007. Hypericaceae. En K. Kubitzki (Ed), *The families and genera of vascular plants*: (pp. 194–201). Berlin, Alemania: Springer Verlag.

Vizcaya, M., Morales, A., Rojas, J. and Nuñez, R. 2012. Revisión bibliográfica sobre la composición química y actividades farmacológicas del género *Vismia* (Guttiferae). BLACPMA 11: 12–34.

Vizcaya, M., Pérez, C., Rojas, J., Rojas-Fermín, L., Plaza, C., Morales, A. and Pérez, P. 2014. Composición química y evaluación de la actividad antifúngica del aceite esencial de corteza de *Vismia baccifera* var. *dealbata*. Revista de la Sociedad Venezolana de Microbiología 34: 86–90.

Tala, M.F., Krohn, K., Hussain, H., Kouam, S.F., Wabo, H.K., Tane, P., Schulzd, B. and Hu, Q. 2007. Laurentixanthone C: A new antifungal and algicidal xanthone from stem bark of *Vismia laurentii*. Z. Naturforsch. 62b: 565–568.

Tala, M.F., Tamokou, J.D., Tchakam, P.D., Tane, P., Kuiate, J.R. and Wabo, H.K. 2011. Antioxidant xanthones, anthraquinoes and semi-synthetic derivatives from *Vismia rubescens* and *Vismia laurentii*. Pharmacologyonline 3: 1410–1418.

Tamokou, J., Tala, M., Wabo, H., Kuiate, J. and Tane, P. 2009. Antmicrobial activities of methanol extract and compounds from stem bark of *Vismia rubescens*. J. Ethnopharmacol. 124: 571–575.

Tchamo, D., Brelot, M., Antheaume, C., Ngouela, S. and Lobstein, A. 2008. Methyl 5,7–dihydrohy-2, 2,9–trimethyl-6,11–dihydro-2H –anthra-[2,3-b] pyran -8- carboxylate. Acta Crystallogr. 64: 2414–2415.

Trópicos org. (Estados Unidos). Missouri Botanical Garden [en línea]: *catálogo automatizado*. [Saint Louis]: Missouri Botanical Garden. <http://www.tropicos.org/Home.aspx> [Consulta: 28 de abril de 2015].

Wabo, H.K., Kouam, S.F., Krohn, K., Hussain, H., Tala, M.F., Tane, P., Ree, T., Hu, Q. and Schulz, B. 2007. Prenylated anthraquinones and other constituents from the seeds of *Vismia laurentii*. Chem. Pharm. Bull. 55:1640–1642.

Wink, M. 2007. Bioprospecting: The search for bioactive lead structures from nature. pp. 97–116. *In*: Kayser, K. and Quax, Q. (eds.). Medicinal Plant Biotechnology. From basic research to industrial application. Weinheim, Alemania: Wiley-VCH Verlag GmbH & Co. KGaA.

8

Curcuma longa L.: From Ethnomedicinal to Novel Biomedical Applications

Mahendra Rai,[1,*] *Avinash Ingle,*[1] *Raksha Pandit,*[1] *Priti Paralikar,*[1] *Farkhanda Rehman,*[1] *Netravati Anasane,*[1] *Pramod Ingle*[1] and *Suneesh Buxy*[2]

Introduction

Turmeric is a medicinal plant, which is also known as "golden spice" and "spice of life". It has been utilized as a medicinal plant and treated as sacred from the ancient Vedic period. The spice turmeric is derived from rhizome of *Curcuma longa,* which is a perennial herb, belonging to the Zingiber family (Fig. 8.1). It is originated in India and is cultivated in tropical and subtropical regions of the whole world (Kojima et al. 1998). Curcumin is the natural, most active polyphenolic compound present in turmeric. Curcumin is the most studied compound of turmeric because of its therapeutic properties. Curcumin is crystalline, orange-yellow and the color of turmeric is mainly due to the presence of curcumin in *Curcuma longa.* The rhizome of turmeric is documented as "herb of the sun" by the people of the Vedic period. Turmeric was used as medicine from at least 6000 years ago. It is most extensively cultivated in India, Bangladesh, China followed by Malaysia, Thailand (Kojima et al. 1998, Shishodia et al. 2005). The use of turmeric, known as "Haridra" was first time stated in 'Arthavaveda' and turmeric has been considered as an effective drug for skin diseases, graying of hair, etc. Even in Tibetan medicine the term "Haridra" means turmeric (Nadkarni 1976). Turmeric is one of the important spice which add flavors and color to food. In the middle-east, Iran is a major importer of turmeric. The Food and Agriculture Organization (FAO) of United Nations projected that approximately 5000 tons of turmeric is imported annually to Iran. The consumption of curcumin is increasing in Iran (Nadkarni 1976, Asgharil et al. 2009).

By using ethanol and acetone, curcumin can be isolated from turmeric and the chemical analysis of curcumin comprised of carbohydrates (69.4%), moisture (13.1%), protein (6.3%), fat (5.1%), and minerals (3.5%). The bioactive components of turmeric is divided into two types: volatile compounds and non-

[1] Department of Biotechnology, SGB Amravati University, Amravati-444602, Maharashtra, India.
[2] Deputy Inspector General (Research and Training), Ministry of Environment, Forest and Climate Change, Government of India.
* Corresponding author: mahendrarai@sgbau.ac.in; pmkrai@hotmail.com

Fig. 8.1 Turmeric plant.

volatile compounds. Monoterpenoids and sesquiterpenoids, such as germacrone, elemene, furanodiene, furanodienone, curcumol, curdione, curcumenol, curzerene, camphor, germacrene B, germa- crene D, isocurcumenol, α-phellandrene, sabinene, cineol, borneol, zingiberene, and sesquiterpenes and neocurdione are different volatile components of *Curcuma longa* (Asghari et al. 2009, Lu et al. 2012). Essential oil from *Curcuma longa* is responsible for its potential biological activities. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin, are the major non-volatile compounds of *Curcuma longa*. The concentration of volatile compounds is more as compared with non-volatile compounds (Fujisawa et al. 2004). It was reported that essential oil composition, biomass production and curcuminoid composition all are affected by the cultivation conditions such as growth media, climatic conditions and nutrient availability (Asghari et al. 2009).

History

Vogel and Pelletier about 200 years ago, defined curcumin as "substance that gives the yellow color". The chemical structure was determined by Roughley and Whiting in 1973. The melting point of curcumin is 176–177°C. In the mid 1900s, curcumin was known as an active component, which exhibited antimicrobial activity against *Staphylococcus aureus, Salmonella paratyphi, Mycobacterium tuberculosis,* and *Trichophyton gypseum,* etc. In the last 50 years, it was found that curcumin has the potential effect on various diseases such as diabetes, allergies, arthritis, Alzheimer's disease, diabetic wounds, hepatic disorders, rheumatism and sinusitis, cough and biliary disorders (Nanjwade et al. 2015). Curcumin can also be used in disorders related to abdominal pain, etc. Ethnologically curcumin occupies a major role in India and it has important position in Indian food. Even in religious ceremonies, the use of turmeric has importance (Shrishail et al. 2013).

From more than 2500 years, turmeric is known for its medicinal treatment in Asian countries. It is stated in 'Ayurveda' and traditional Chinese medicine that curcumin has many advantages in the prevention and treatment of many diseases. In old Hindu texts, curcumin is ascribed for its aromatic, carminative, stimulant properties. When turmeric is mixed with slaked lime, it is used in the treatment of sprains and swellings caused by injury. Curcumin has many household applications as it can be applied on the locally affected area of wound infection and other chronic illness (Hermann and Wahl 1991).

In Ayurveda, turmeric is known for its wide range of applications. Curcumin can be used to treat flatulence, dyspepsia, liver disorders (jaundice in particular), eye and ear infections, small-pox, chicken pox, common cold, ulcers and inflammation, intermittent fevers, eczema, sprain, bruises, wounds, inflammatory troubles of joints, small pox, chicken pox, catarrhal and purulent ophthalmic, conjunctivitis, ring worm, etc. (Hermann et al. 1991, Rai et al. 2015). Curcumin is known to have a cholesterol-lowering capacity, antidiabetic, anti-inflammatory, and antioxidant properties and to have an anticancer activity. From the

clinical trials with humans, it was determined that curcumin was safe and effective. The Food and Drug Administration (FDA) acknowledged curcumin as a compound "generally recognized as safe" (Pulido-Moran et al. 2016).

Derivatives of curcumin

Curcumin is also known as diferuloylmethane and its chemical formula is $C_{21}H_{20}O_6$. Chemical denotation of curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]. Curcumin is not soluble in water at acidic or neutral pH. However, it is soluble in organic solvent such as acetone, methanol, and ethanol. It is revealed that curcumin is highly sensitive to light and, therefore, it is suggested that biological samples containing curcumin are to be protected from light (Pulido-Moran et al. 2016). Curcuminoid complexes are present in the rhizome of turmeric and it comprises of curcumin (Curcumin I), demethoxycurcumin (Curcumin II) and bisdemethoxycurcumin (Curcumin III). Demethoxycurcumin and bismethoxycurcumin are the derivatives of curcumin in the rhizome of *Curcuma longa,* along with curcumin's two derivatives present in the rhizome of *Curcuma longa*. The commercially available curcumin contains Curcumin I (77%), Curcumin II (17%) and Curcumin III (3%), etc. (Lee et al. 2013). The physicochemical properties of curcumin and its derivatives are given in the Table 8.1.

Bioactivities of curcumin

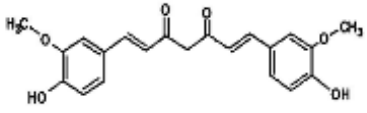
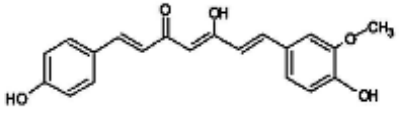
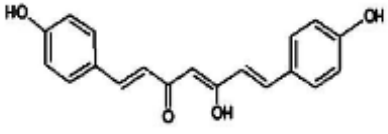
Curcuma longa has been attributed to numerous bioactivities including antimicrobial, anticancerous, antioxidant, anti-inflammatory, etc. During the span of 60 years, more than 3000 studies have been reported on various bioactivities of curcumin and its existing benefits in medicine for treatment of allergy, cardiovascular diseases, lung fibrosis, neurodegenerative diseases, inflammatory bowel diseases, arthritis, etc. (Aggarwal and Harikumar 2009, Chandran and Goel 2012). Some of the bioactivities of curcumin are illustrated below.

Anti-inflammatory activity

Curcuma longa has been attributed to numerous bioactivities including antimicrobial, anticancerous, antioxidant, anti-inflammatory, psoriasis, etc. Traditionally, *Curcuma longa* has been used in anti-inflammatory treatment in Ayurvedic medicine (Goel et al. 2008). The anti-inflammatory effect of curcumin was very well reported in clinical as well as experimental studies (Menon and Sudheer 2007, Ferreira et al. 2015, Zhang et al. 2015, Ma et al. 2017). The anti-inflammatory effect of curcumin is mediated through enzymes which has the ability to inhibit cyclooxygenase-2 (COX-2), Nitric Oxide Synthase (NOS) and lipoxygenase (LOX). These enzymes are important for inflammatory processes (Menon and Sudheer 2007, Aggarwal and Harikumar 2009, Basnet and Skalko-Basnet 2011). Chainani-Wu (2004) reported the study of anti-inflammatory activity of curcuminoid which is the main constituent of curcumin on humans. The study revealed that curcumin exerts effective anti-inflammatory activity by inhibiting different molecules which includes lipoxygenase, tumor necrotic factor, interleukin-12, phospholipase, collagenase and interferon inducing proteins. Recently, Ullah et al. (2017) reported curcumin as a therapeutic option to limit neuroinflammation in neurodegenerative disorders. Pharmacokinetic studies of curcumin have provided the information about the bioavailability of curcumin in targeted tissues which implicate potential therapeutic effect on neuroinflammation.

Microglia mediated neuroinflammation is an important agent in brain hemorrhage to inflammatory injury. The study carried out by Yang et al. (2014) showed that curcumin inhibits the inflammatory cytokinine production in brain hemorrhage induced BV2 microglial cells. Similar studies were also reported by Cheng et al. (2001), which showed that curcumin inhibits LPS induced neuroinflammation and cytokine production in BV2 cell line. The study revealed that pretreatment BV2 microglia cells with curcumin resulted in microglial inflammation reduction and decrease in cytokine production in a dose dependent manner. Curcumin analogues namely diarylpentadienone have anti-inflammatory activity against LPS induced interleukin released in RAW264.7 cell (Wang et al. 2017). It was found that curcumin analogues

Table 8.1 Physicochemical properties of curcumin and its derivatives.

Structure	Common name	Chemical name	Molecular mass (g/mol)	Melting point (°C)	Solubility in alcohol/ acetone
	curcumin	Di-cinnamoylmethane	368.4	183–186	Soluble
	demethoxycurcumin	4-hydroxycinnamoulmethane	338	172–174	Soluble
	bismethoxycurcumin	Bis-4-hydroxycinnamoylmethane	308	224	Soluble

inhibit interleukin-6 and tumor necrosis factor alpha (TNF-α) which showed the highest inflammatory activity. The study also indicates that anti-inflammatory activity of curcumin may be due to NF-kB signaling pathway inhibition. Furthermore, the anti-inflammatory effect of curcumin was also reported to have significant activity against *Helicobacter pylori* infection in mucosal disease (Santos et al. 2015). Curcumin was found to play an important role in respiratory diseases caused by inflammation (Kurup and Barrios 2008). Studies on acute allergic asthma in mice showed that curcumin can act as an anti-inflammatory agent by down regulating Notch1-GATA3 signaling pathway (Chong et al. 2014). Curcumin was also reported as a scavenger of nitric oxide and decreased nitic oxide synthase activity, which could prevent bronchial inflammation in asthmatic patients (Nilani et al. 2009). Due to all its anti-inflammatory properties, curcumin is able to treat various chronic diseases, autoimmune, cardiovascular, endocrine and neurodegenerative diseases.

Wound healing activity

Curcumin is very well known for its wound healing potential because of its antioxidant, anti-infectious and anti-inflammatory properties (Meng et al. 2013, Mun et al. 2013, Akbik et al. 2014). The important role of any wound healing agent is to protect the wound tissue from infection, reduce inflammation and induce reconstruction of damaged tissues (Kulac et al. 2013). Curcumin has also been found to boost the process of wound healing via tissue remodeling, formation and collagen deposition (Akbik et al. 2014). It was also observed that application of curcumin on wounds also accelerate epithelial tissue regeneration, fibroblast proliferation and vascular density (Thangapazham et al. 2013). Jagetia and Rajanikant (2012) reported that curcumin enhances the nitric oxide production, which promotes wound healing in mice. Emiroglu et al. (2017) reported the effect of curcumin on healing of nasal mucosal wounds in mice. The study showed that curcumin reduces the inflammatory response and accelerates wound healing in mice.

Various clinical research conducted by different groups revealed that curcumin accelerates the wound healing process by decreasing our body's natural reaction such as inflammation towards the wound (Gupta et al. 2013, He et al. 2015). Curcumin is also reported to improve wound healing due to its free radical scavenging activity. It was also reported that antioxidant property of curcumin is due to its hydroxyl and methoxy groups (Rahman and Biswas 2009). The introduction of curcumin for targeting Reactive Oxygen Species (ROS) to the wound site could be a vital approach to eliminate ROS and improve healing at the wounded area (Mohanty and Sahoo 2017). Many studies showed that curcumin exerts different roles on different wound healing stages. At the inflammation phase, curcumin was notably reported to inhibit the activity of NF transcription factor, also inhibiting the production of TNF-α, IL-1 cytokines which reduce the inflammation at the wounded site (Akbik et al. 2014). Joe et al. (2004) explained numerous mechanisms by which curcumin exerts wound healing activity via various processes. Most importantly, curcumin was reported to modulate inflammation in the wounded site by inhibiting the production of TNF and interleukins which plays an important role in inflammatory response regulation. Curcumin was also equally important to inhibit activity of nuclear factor kappa-light-enhancer of activated B cells (NF-(κ) B). NF-(κ) B is a transcription factor that regulates many genes which are implicated in the inflammatory response. Curcumin affects a variety of pathways implicated in activation of NF-(κ) B (Joe et al. 2004). Curcumin was also reported to show enhanced fibroblast migration, tissue formation and collagen deposition at the wounded site (Blakytny and Jude 2006, Loughlin and Artlett 2011, Mohanty et al. 2012). Similarly, curcumin has the ability to improve wound contraction by increasing the production of TGF-β which ultimately increases fibroblast proliferation (Akbik et al. 2014). This property of curcumin reduces the time needed for wound healing.

Anticancerous activity

Curcumin also possesses anticancer properties due to its ability to inhibit cell proliferation and promotes apoptosis. Curcumin exerts its bioactivity at different stages of carcinogenesis which includes, prevention of cancer related inflammation, inhibit oncogene activation and cell proliferation, induces apoptosis and prevents metastasis (Pongrakhananon and Rojanasakul 2011). The influence of curcumin on cancer cells is

illustrated in Fig. 8.2. The anticancerous effect of curcumin depends on the concentration, time of treatment, dose and cell type. At lower concentration, curcumin were reported to cause cell cycle arrest while, at higher concentration it induces apoptosis (Kastan and Bartek 2004, Pongrakhananon and Rojanasakul 2011).

Curcumin has induced apoptosis in a number cell lines including melanoma, leukemia, cancerous cell line of breast, colon, lungs, ovaries and the kidneys which leads to cancer cell death via mitochondrial death pathway (Karunagaran et al. 2005). Yang et al. (2013) demonstrated that curcumin significantly reduces the levels of CDK4 and cyclin D1, which are cell cycle regulators and suppressed the expression of p53 in the mouse colon. The study also revealed that curcumin blocks STAT3 signaling by provoking the anti-inflammatory effect. Curcumin was also reported to induce stress in endoplasmic reticulum and cause apoptosis in tumor cells (Vallianou et al. 2015). Curcumin was also effectively used for treatment of melanoma. The study carried out by Jiang et al. (2015) suggested that curcumin alters the expression levels of p53, p38, NF-(κ)B which is associated with apoptosis and also inhibits proliferation of melanoma cells in human. Curcumin was also reported to significantly inhibit the gastric tumor cell growth via ROS generation, which results in depletion of mtDNA, ultimately disrupts the cellular bioenergetics (Wang et al. 2017).

Apart from the apoptosis inducing effect, curcumin also possesses chemopreventive ability to stabilize cancer cells for chemotherapy induced cell death. Generally, the problem associated with cancer chemotherapy is the cytotoxicity to normal cells and acquiring of apoptosis resistance of cancer cells. For this, the combinational therapy approach is preferred to reduce the cytotoxicity using optimal dose regimens. Curcumin is one of the agents which has been used for its effect in combination therapy. Javvadi et al. (2008) reported curcumin as a chemopreventive agent and potent radiostabilizer of human cervical carcinoma cells. The study showed that pretreatment of curcumin to HeLa and SiHa, a cervical carcinoma cell lines before radiation therapy results in effective dose dependent radio stabilization of cervical carcinoma cells. Quian et al. (2015) reported that curcumin enhances radio sensitivity of glioma U87 human cells by upregulating the DUSP-2 expression and decreases the phosphorylation of ERK and JNK. The study also revealed that curcumin inhibits U87 glioma human cell proliferation in a time and dose dependent manner. Curcumin was reported as a potential anticancer agent. It is also gaining huge acceptance due to its various bioactivities.

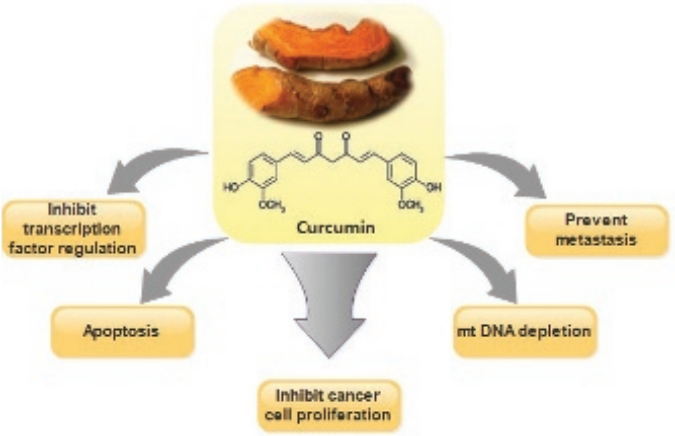


Fig. 8.2 The influence of curcumin on cancer cell.

Curcuma longa cures dermatological diseases

The varied biological properties and lack of toxicity even at higher doses of curcumin is one of the most important content of *Curcuma longa,* which makes it attractive to explore its use in various disorders including dermatological diseases. Dermatological diseases are diseases of skin which affect many people. Skin is the first line of defense and is vulnerable to various adverse conditions like burns, injuries, infections and disorders like acne, hyper or hypo pigmentation, dermatitis, psoriasis and cancer (Thangapazham et al. 2007).

Acne

Acne is a chronic inflammatory disease of hair follicles (pilosebaceous unit) resulting from androgen-induced increased sebum production, altered keratinisation, inflammation, and bacterial infections on the face, neck, chest and back by *Propionibacterium acnes.* In addition to *P. acnes*, as the main causative microorganism, *Pityrosporum ovale* and *Staphylococcus epidermidis* are also present in acne lesions (Kanlayavattanakul and Lourith 2011, Williums et al. 2012). According to a study led by Lalla et al. (2001) use of oral as well as topical preparations of *Curcuma longa* showed better efficacy than taking internal preparations alone to treat acne. *Curcuma longa* along with two more herbs *viz. Azadirachta indica* and *Hemidesmus incidus* when used in acne treatment, led to inflammation being significantly reduced and suppressed the reactive oxygen species produced by *P. acnes* (Jain and Basal 2003).

Atopic dermatitis

Atopic dermatitis (eczema) is a medical condition characterized by inflammation, extreme dryness, itching, redness, scaly patches and irritation of the skin. It is found in people who have a tendency to develop allergic reactions to certain external compounds. It is commonly found in association with allergic rhinitis, asthma or other symptoms of atopy. The inflammatory symptoms in different organs are observed due to the release of leukotrienes, prostaglandins and proteases (Zari and Zari 2015). The active ingredient curcumin present in *C. longa* shows anti-inflammatory and bactericidal property. It lowers the expression of inflammatory enzymes in the body and treats skin inflammation associated with eczema. Curcumin inhibits the occurrences of this disease by reducing the sensitivity of some inflammatory transcription factors like NF-κB, cytokines like TNF, IL-1 and IL-6, some enzymes like cyclo-oxygenase 2 or 5-lipoxygenase. The herbal paste of *C. longa* (turmeric powder mixed with sweet lime juice and salt) is applied on swellings. This paste provides rapid and long lasting relief. It treats wounds and stops bleeding (Khiljee et al. 2011, Zari and Zari 2015).

Facial photoaging

Facial photoaging occurs due to excessive exposure to the sun as early as the second decade of life. Ultraviolet (UV) radiation viz. UVC, UVB and UVA causes mutations by generating reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radical and increases the expression of matrix metalloproteinases in skin connective tissues and dermal layers of the skin which degrades skin collagen and contributes to photoaging. The degradation of endogenous type I collagen fibrils was increased by 58% in the UV irradiated skin (Cleaver 1968, Kadekaro et al. 2003, Aoki et al. 2007). A decreased collagen production in dermal fibroblasts and a subsequent fragmentation and disarray of dermis collagen fibrils are also features of age-related phenotypic alterations in human skin (Satoh 1992, Taylor 2002, Takashahi et al. 2017). In a study, the use of a herbal gel formulation Tricutan® containing *C. longa*, *Rosmarinus officinalis* and *Centella asiatica* was evaluated in 28 women (aged 34–37 years) suffering with photoaging which showed significant improvement in skin firmness and clinical evaluations when applied for 4 weeks (Sommerfeld 2007).

Psoriasis

Psoriasis is a chronic, hyper proliferative, inflammatory and non-contagious skin disease caused by faulty signals in the immune system and is generally considered an autoimmune disease mediated by T-cells (Kastelan et al. 2006). It causes thick, whitish flakes of scale on raised pinkish red or black skin with well-defined margins. Curcumin, the key component of *C. longa* extract has the ability to be developed as an antipsoriatic drug because it reduces keratinocyte proliferation and was found to be effective in the mouse tail animal model of psoriasis (Bosman 1994, Pol et al. 2003). Curcumin reduces the expression of pro-inflammatory cytokines like IL-6 and IL-8 in human keratinocytes. These cytokines are both pro-

inflammatory and are growth factors for keratinocytes. Hence, their inhibition by curcumin might reduce inflammation and keratinocyte hyperproliferation caused by psoriasis (Miquel et al. 2002).

Topical therapies such as glucocorticoid treatment is effective due to its anti-inflammatory property but discontinuation is encouraged due to its side effects (Lebwohl 2004). Therefore, suitable alternatives are important for treating psoriasis. The rhizome of *C. longa* has a beneficial effect on psoriasis due to the polyphenolic compound curcumin and antioxidants present in it. Other than topical therapies, phototherapy is also considered effective and safe for treating psoriasis. Curcumin has been proved as phototoxic for *Salmonella typhimurium* and *Escherichia coli* on irradiation with visible light (Tonneson et al. 1987). This potential photosensitizing principle of curcumin makes it applicable in the phototherapy of psoriasis. The combination of phototherapy with curcumin might accelerate the clinical response and might even diminish the exposure load (Thangapazham et al. 2007, Miguel et al. 2015).

Pruritus

Pruritus or simply itching is a restless sensation and emotional ordeal linked to a skin disturbance that induces the desire to scratch. It can result from external compounds, many systemic diseases and medications (Davidson and Giesler 2010). *C. longa* has been proven effective in treating pruritus (Vyas 2015). In a study, a topical formulation containing 16% turmeric along with other herbal ingredients, was used in 64 patients of both sexes, with pruritus caused by multiple etiologies (atopic dermatitis, senile pruritus, and ichthyosis). A significant improvement was observed in the patients with pruritus (Chatterjee et al. 2005).

Oral Lichen Planus

Lichen planus is a chronic inflammatory disease which affects the mucosal and cutaneous tissue. Lichen planus may appear in the esophagus, mouth, vaginal mucosa or skin but mostly it is found in the oral cavity. Often, it is found only in the oral cavity. Oral Lichen Planus (OLP) occurs more frequently than the cutaneous form and tends to be more resistant to any treatment. Overall, lichen planus affects approximately 2% of the population. This disease most commonly affects women over the age of 50 however, it may occur in all age groups. The exact cause of this immune mediated basal cell damage is unknown but it is believed that it occurs due to an abnormal T-cell mediated immune response. It is often asymptomatic; the atrophic erosive form can show symptoms like a burning sensation and severe pain, which makes it difficult to speak, eat and swallow. Patients with these symptoms require treatment. Plaque and calculus deposits are associated with significantly higher incidence of redness and erosive gingival OLP. Good oral hygiene is essential and it enhances the healing process (Edwards and Kelsch 2002, Lodi et al. 2005).

A pilot study was conducted in the department of oral and maxillofacial surgery for which 10 patients were clinically diagnosed and histopathologically confirmed as patients of OLP. The rhizome extract of *C. longa* in the ointment form was made at NBRI, used for local application twice/day for a period of 3 months. The extract showed beneficial effects on patients and proved to be a better alternative to other steroidal drugs, which shows hazardous side effects (Singh et al. 2013).

Vitiligo

Vitiligo is a skin disease in which the melanocytes (pigment producing) cells are destroyed, resulting in white patches on the skin of different parts of the body. Although the exact cause of the damage to melanocytes is not clear, oxidative stress has been associated in the pathogenesis of the disease (Schallreuter et al. 1996). Narrowband UVB (NB-UVB) that uses the portion of the UVB spectrum from 311 to 312 nm is considered as the gold standard treatment for vitiligo (Arca et al. 2006). Because of the anti-oxidant property of curcumin, it seems to be a therapeutic option for the treatment of vitiligo. One study investigated whether the combination of NB-UVB and tetrahydro-curcuminoid cream could result in synergistic therapeutic effects against vitiligo (Asawanonda and Klahan 2010). Ten patients with vitiligo were enrolled in the study. Two similar lesions were treated with either NB-UVB plus topical tetrahydro-curcuminoid cream

or with UVB alone. Results indicated a statistically significant repigmentation in both treatment groups with slightly better repigmentation in the combination group (Asawanonda and Klahan 2010).

Curcumin application in Neurological Disorders

Parkinson's Disease

Parkinson's Disease (PD) is characterized by neurodegeneration of dopaminergic neurons from *Substantia Nigra* (SN) part of the brain. This neurodegeneration results in reduced dopamine secretion causing tremors, depression and bradykinesia which primarily affect body and brain coordination (Jankovic, 2008). PD is characterized by selective loss of dopaminergic neurons and accumulation of α-syn based and other proteins in Lewy bodies (Dauer and Przedborski 2003, Dawson and Dawson 2003). Formation of reactive species like peroxynitrite (PN) (Mythri et al. 2011), toxic aldehydes such as 4HNE and MDA (Yoritaka et al. 1996) mainly affects the biomolecules, thus damaging the cellular machinery, e.g., inhibition of brain mitochondrial complex I (CI) activity and reducing mitochondrial membrane potential and integrity. Rajeswari and Sabesan (2008) studied the inhibitory effect of polyphenolic curcumin and its metabolite tetrahydrocurcumin on monoamine oxidase-B MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced PD in model mice. Both curcumin (80 mg/kg i. p.) and tetrahydrocurcumin (60 mg/kg i. p.) served as a neuroprotectant against MPTP-induced depletion of DA and DOPAC (Rajeswari and Sabesan 2008). Kundu and colleagues (2016) studied the anti-Parkinson activity of curcumin and piperine coloaded glyceryl monooleate (GMO) nanoparticles encoated with various detergents pertaining higher bioavailability in rotenone induced PD mice (Szczepanowicz et al. 2016). Mythri et al. (2011) have shown that the regular consumption of curcumin provides neuroprotection in toxic mouse model of PD. They have studied the neuroprotective property of different conjugates of curcumin, among which enhanced protection against PN-dependent Mitochondrial Complex I inhibition of dopaminergic neurons (Mythri et al. 2007) and protein nitration was shown by glutamoyl diester of curcumin as compared to other conjugates. Di-glutamoyl curcumin protected dopaminergic neurons against 1-methyl-4-phenylpyridinium (MPP)-mediated neuronal death (Mythri et al. 2011). The antioxidant activity of curcumin protects SN neurons, improves striatal dopamine levels and chelates Fe^{2+} in the 6-OHDA rat model of PD (Jiao et al. 2009). This is probably mediated by the phenolic rings and diketone groups of curcumin which serve as an electron trap thus preventing formation of H_2O_2, OH^- and superoxide (Zbarsky et al. 2005). Szczepanowicz et al. (2016) synthesized multilayered Poly-L-Lysine (PLL) and Poly-L-Glutamic Acid (PGA) encapsulated curcumin nanocarriers for the prevention of H_2O_2-induced reduction in mitochondrial membrane potential in SH-SY5Y human neuroblastoma cell line (Szczepanowicz et al. 2016). Interestingly, curcumin treatment restores lipid peroxidation mediated mitochondrial damage and apoptosis (Zhu et al. 2004). Curcumin protects against 4HNE mediated alterations in mitochondrial respiratory function and redox metabolism, cytochrome C release, DNA fragmentation and PARP activation in PC12 cells demonstrating a protective function against lipid peroxidation mediated damage (Raza et al. 2008). Pretreatment with curcumin reestablishes mitochondrial membrane potential, elevates in Cu/Zn SOD and modulates NFκB nuclear translocation (Wang et al. 2009), inhibits IL-6 and TNF-alpha (Wang et al. 2009), aggregation of α-synuclein *in vitro* (Pandey et al. 2008, Wang et al. 2010) and reduction in aggregation of synphilin-1 (Pal et al. 2011). Curcumin also activates proteins involved in iron metabolism in SN of PD brains (Sofic et al. 1988), thus suppressing levels of ferritin and hepcidin, representing its role as an iron chelator (Jiao et al. 2006). Curcumin treatment thus increases the density of dopaminergic neurons in the SN (Vajragupta et al. 2003) possibly due to neurogenesis (Xu et al. 2007, Kim et al. 2008).

Alzeimer's Disease

Alzheimer's Disease (AD) is a devastating neurodegenerative, persistent and progressive disorder. Amyloid Proteins (AP) are necessarily required for the brain's normal synaptic function (Hardy and Selkoe 2002). AD is found to be the most common cause of dementia in 50–60% of all dementia cases (Blennow et al. 2006). There is an increase in the production of amyloid protein (Aβ) aggregates which forms plaques which are feebly cleared from the system (Ghiso and Frangione 2002, Mawuenyega et al. 2010, Mathew

et al. 2011). AP degradation in the normal brain is a result of various enzymes (Iwata et al. 2005). AD was initially elucidated by German psychiatrist Alois Alzheimer (1864–1915) in 1906 and was named by Kraepelin (Blennow et al. 2006). Ahmed and Gilani (2009, 2014) reviewed the neuroprotective property of each component of the curcuminoid mixture that plays an important role in AD representing curcuminoid mixture better than the curcumin. It has been observed from the epidemiological studies the consumption of turmeric (curcumin) by Asian Indians is perhaps the reason for the low occurrence of AD/PD as well as 40% less melanized nigral neurons in brains in India as compared to the Caucasians (Muthane et al. 1998, Blennow et al. 2006). Pan et al. (1998) demonstrated that curcumin is the only natural compound that can cross the blood-brain barrier, but only in traces. Begum et al. (2008) and Aggarwal and Harikumar (2009) studied the methods of enhancing the bioavailability of hydrophobic curcumin using different approaches. Ringman and colleagues (2005) examined the dietary curcumin for their properties like antioxidant, anti-inflammatory, or anti-amyloidogenic on mice model, either administered chronically to aged Tg2576 APPsw mice or acutely to lipopolysaccharide (LPS)-injected wild-type mice. In both the cases, reduction in interleukin-1β was reported. It was concluded that the curcumin is promisingly effective in reducing amyloid plaque burden, insoluble β-amyloid peptide (A β), and carbonyls (Ringman et al. 2005). Wang and colleagues (2008) have demonstrated the degrees of saturation, types of carbon skeleton and functional group, and hydrophobicity plays an important role in anti-BACE-1 and behavioral activities of curcuminoids and their derivatives from rhizomes of *Curcuma longa* (Zingiberaceae) (Wang et al. 2008). Curcumin has proved its role as neuroprotective agent in models of stroke and AD (Lim et al. 2001, Yang et al. 2005, Ringman et al. 2005). Yang et al. (2005) have confirmed inhibitory action of curcumin on amyloid beta (Aβ) oligomers and fibrils in AD. Lim and colleagues (2001) reported the reduction in amyloid pathology, oxidative stress and pro-inflammatory markers in transgenic AD mice model. In addition, curcumin reduces the levels of Glial Fibrillary Acidic Protein (GFAP), a marker of astrocytic proliferation. Deposition of amyloid β-peptide and markers of oxidative stress and inflammation was found to be reduced in cerebral cortex of model AD mice after dietary supplementation of curcumin (160–5000 ppm) (Lim et al. 2001). Mathew et al. (2012) demonstrated that amyloid-binding aptamer (named NN2) conjugated curcumin–poly (lactic-co-glycolic acid) (PLGA) nanoparticle can be used as an effective plasma amyloid level minimizer, as it binds and deteriorates amyloid plaques. Over-expression of curcumin induced RANTES, a chemokine released by astrocytes and microglia plays a key role in neuroinflammatory pathways during AD leading to enhanced neuronal survival (Lin et al. 2011). Cheng and coworkers (2013) have formulated stable curcumin nanoparticles to test *in vitro* and in AD model Tg2576 mice. This nanocurcumin produced significantly higher curcumin concentration than normal curcumin in plasma and better working memory in models. Curcumin loaded polybutylcyanoacrylate nanoparticles (PBCN) encoated with polysorbate 80 were formulated by Sun and coworkers (2010). The pharmacokinetic and bio-distribution of this nanoparticle formulation was studied by intravenous administration in the tail vein and crossing across the blood-brain barrier was observed (Sun et al. 2010).

Curcumin in medicine

Curcumin is a multi-functional and pharmacologically safe natural agent (Paul et al. 2016) and is extensively used as an antibacterial, antifungal, antiviral as well as antiparasitic agent from ancient times (Morais et al. 2013, Tyagi et al. 2015, Teow et al. 2016). It inhibits growth of microbes by various routes such as inhibition of cytokinesis and cell proliferation, increasing sensitivity of microbes towards antibiotics and perturbation of the cell wall and membrane leading to cell lysis, inhibiting arachidonic acid metabolism, stabilization of lysosomal membrane which causes uncoupling of oxidative phosphorylation, modulation of different transcription factors, cytokines, enzymes, receptors, etc. (Shanmugam et al. 2015, Paul et al. 2016, Teow et al. 2016). Lethal effect of curcumin on microbes can be evaluated by different assays such as killing assay, membrane permeabilization assay, propidium iodide uptake assay, calcein leakage assay, scanning electron microscopy, Plasma Membrane Fluorescence Intensity measurement, etc. (Lee et al. 2014, Tyagi et al. 2015). Different antimicrobial and antiparasitic effects studied so far are summarized in the Table 8.2.

Curcumin, with its wide range of functions, is helpful in a variety of medical conditions. Rheumatoid arthritis, is a condition in which inflammation leads to immunological reactions followed by tissue damage at joints, occurrence is heightened with increased age (Kumar and Rai 2011, Johnson and Hunter 2014, Daily

Table 8.2 Antimicrobial and antiparasitic efficacy of curcumin.

Biological activity	Type of curcumin	Test organism	Mechanism	References
Antibacterial activity	Curcumin	*S. aureus, E. coli*	Inhibition of biofilm production	Kali et al. 2016
	Curcumin I	*S. aureus, E. faecalis, E. coli, P. aeruginosa*	Cell death by damaging membrane	Tyagi et al. 2015
	Curcumin, Bisdemethoxy-curcumin, Demethoxycur-cumin	*B. subtilis, K.pneumoniae, E. coli, E. aerogenes, P. aeruginosa, S. aureus*, *P. mirabilis*		Singh and Jain 2012
	Curcumin	*S. mutans, Actinomyces viscosus, Lactobacillus casei, Porphyromonas gingivalis*, *E. faecalis Prevotella intermedia*		Mandroli and Bhat 2013
	Curcumin + cefaclor, cefodizime, and cefotaxime	*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Vibrio cholerae*		Sasidharan et al. 2014
Antifungal activity	Curcumin	*A. niger, C. albicans*		Singh and Jain 2012
	Curcumin	*C. albicans, C. parapsilosis, C. tropicalis, C. dubliniensis*	Inhibits adhestion of Cells to BEC (human buccal epithelial cells)	Martins et al. 2009
	Curcumin	*C. albicans*	Triggers early apoptosis, Inhibits Hyphal development by regulating *TUP1* levels	Sharma et al. 2010
Antiviral activity	Curcumin	Human influenza virus Avian influenza virus	Reduces influenza A viruses replication, Blocks haemagglutinating activity of IAV virus particles affects an early stage of virus infection	Chen et al. 2010
	Curcumin	Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2)	Inhibits adsorption of virons on cells	Flores et al. 2016
Anti HIV activity	Curcumin	HIV	Inhibits UV induced HIV gene expression	Taher et al. 2003
	Curcumin	HIV	Inhibits HIV-1 integrase required for viral replication	Mazumdar et al. 1995, De Clercq 2000
Antiparasitic activity	Curcumin	*Schistosoma mansoni*	Transcriptional repression in Notch and TGF pathways, Affects oviposition and egg development	Morais et al. 2013
	Curcumin+ Praziquantel (PZQ)	*Schistosoma mansoni*	Reduce fibrosis, Increases oxidative/ nitrosative stress, fragmentation of nuclear DNA	Aboueldahab and Elhussieny 2016

	Curcumin	*Giardia lamblia*	Antioxidant activity, Affects trophozoite attachment	Sadhana and Gupta 2013, Dyab et al. 2016
	Curcumin	*Giardia lamblia*	Inhibits trophozoites attachment and growth, Destabilizes microtubules	Filiberto et al. 2017
	Curcumin + benznidazole	*Trypanosoma cruzi*	Limits anti-parasitic activity	Novaes et al. 2016
Antimalarial activity	Curcumin + Artemisinin	*Plasmodium berghei*	Generation of ROS/IgG antibodies	Padmanabhan et al. 2012
	Curcumin, Curcumin + chloroquine + piperine, Curcumin + piperine + artemisinin	*Plasmodium chabaudi*	Interference in Ubiquitin Proteasome System, Increases synthesis of deubiquitylating Enzymes	Neto et al. 2013
	Curcumin	*Plasmodium falciparum*	Disruption of Microtubules	Chakrabarti et al. 2013

et al. 2016). Curcumin, shows anti-inflammatory activity by inhibiting enzyme triggering inflammation, such as cyclooxygenase 2 (COX2). By the inhibition of COX2, it eventually reduces prostaglandins synthesis, which mediates signaling of pain (Kumar and Rai 2011).

Alzheimer's Disease (AD), usually observed in later stages of life, is a neurodegenerative disorder involving aggregation of protein Aβ into fibrils and eventually amyloid plaques which deposits into the brain, interfering with memory, thinking and behavior of the patient. Curcumin, with its antioxidant, anti-inflammatory and antiamyloid potential, helps to overcome symptoms of AD, *in vitro* and in animal models. In order to study safety, tolerability, pharmacokinetics, efficacy and effects of curcumin on biomarkers associated with the AD, phase II, randomized, double-blind, placebo-controlled study was performed with 33 patients of AD, some were given low-dose (2 g/day) and another group with high-dose (4 g/day) for 24 weeks. After 24 weeks inspite of its low bioavailability and withdrawal of three patients developing gastrointestinal symptoms, curcumin was well tolerated (Ringman et al. 2012). Another randomized, double-blind, placebo-controlled study was carried out with 34 patients, by giving two different doses 1 g and 4g. Results depict that the mental condition neither improved nor any adverse effect was observed, but it might decrease the need of vitamin E because of its antioxidant activity (Baum et al. 2008). Yao and Xue (2014) from their study observed that curcumin can destabilize formation of A*β* plaque and increase its phagocytosis. It reduces inflammation caused by tau protein and also interacts with cholesterol and metal ions. Curcumin is a potent inhibitor of COX-2 and NF-*κ*B, known for their inflammatory response and hence is a possible remedy for AD through its versatility, with less to no side effects in animal models.

Cardiovascular Disease (CVD) is a condition with impaired functioning of the heart and vascular system (Haldeman et al. 1999, Yach et al. 2004). CVD includes various disease conditions such as persistent hypertension, cardiac inflammation, myocarditis (inflammation of myocardium) and endocarditis (inflammation of endocardium), valvular insufficiency and stenosis, congenital malformations, familial hypertrophic and dilated cardiomyopathies, diabetic cardiomyopathy and myocardial infarction or ischemia associated with coronary artery disease, which are linked to heart attacks. It also involves arterial diseases, e.g., atherosclerosis, inflammatory stenosis and thrombus formation (Jana and Swarnakar 2013). Curcumin holds the ability in reducing risk of CVD by inhibiting the high fat diet related inflammation and hematological changes in the body (El-Habibi et al. 2013). Even in coronary artery disease in humans, curcumin decreases Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), cholesterol and serum triglyceride without any effect on inflammatory biomarkers (Mirzabeigi et al. 2015).

Antiophidian (Antivenom) activity of any biological component can be estimated by its phospholipase A2 (PLA2) inhibition. Snake venom contains PLA2 which exerts effect such as cardiotoxicity, myotoxicity, pre orpostsynaptic neurotoxicity, edema, hemolysis, hypotension, convulsion, platelet aggregation inhibition and anticoagulation (Gutiérrez and Lomonte 1995, Ownby 1998, Teixeira et al. 2009). PLA2 degrades membrane phospholipids, which releases arachidonic acid, precursor of inflammatory compounds such as prostaglandins and leukotrienes (Samy et al. 2012). Docking study of various plant derived compounds including curcumin against Russell's viper and bovine pancreatic PLA2 shows interaction with the Amino Acid (AA) residues at the active site of both PLA2s, shows curcumin of having anti-inflammatory and antidotes properties (Nirmal et al. 2008).

To study the effect of curcumin in gastrointestinal diseases, Holt et al. (2005) conducted a study with five patients diagnosed with Ulcerative Colitis/proctitis (UC) and five patients with Crohn's Disease (CD). UC patients were treated with 5-aminosalicylic acid (5-ASA) + corticosteroids + 550 mg curcumin (twice daily for a month and then thrice daily for the second month). CD patients were given 360 mg curcumin (thrice daily for a month and 360 mg four times daily for the second month). Several hematological, biochemical, and inflammatory analysis and sigmoidoscopy and biopsy depicts that the UC group showed significant improvement followed by reduction in medication and DC group showed improvement with lowered Crohn's Disease Activity Index.

Curcumin, being able to perform inhibition of the pro-inflammatory cytokines NF-κB and IL-6/STAT3 signaling pathway, is useful for the various gastrointestinal inflammatory diseases, such as Inflammatory Bowel Disease (IBD) (Hilsden et al. 2011). Hanai et al. (2006) performed a case study with 89 patients having quiescent UC in a randomized, double-blind, multicenter trial, four week washout period followed by random selection of patients for placebo or curcumin for six months. Curcumin showed improvement in the Clinical Activity Index (CAI) and the Endoscopic Index (EI), well tolerated by patients and have

better clinical efficacy than placebo in the prevention of a relapse. Curcumin showed myorelaxant effect on smooth muscles of the gallbladder, bladder, aorta and trachea and heart. It increased gall bladder and intestinal tone and contraction with no side effects on other organs *in vitro*. Hence, it can be used in various gastrointestinal tract disorders (Micucci et al. 2013).

Limitations of curcumin

Curcumin is a hydrophobic and lipophilic molecule which is not soluble in water. Even though curcumin exhibited potential medicinal properties, there are several limitations demonstrated from pharmacological studies. From the last few years, researchers all over the globe are interested in determining the mechanism of action of curcumin and with this intention many studies with animal and *in vitro* models were studied. For this reason, there are many studies which aim to find out the bioavailability of curcumin (Rai et al. 2015, Saikia et al. 2015). In 1978, Wahlstrom and Blennow for the first time administered curcumin in Sprague-Dawley (1 g/kg dose) to determine biological availability of curcumin (Annon and Wahl 1990). Ryu et al. (2006) demonstrated that when curcumin is intravenously administered in mice, it was accumulated in various organs such as the liver, spleen, lungs and in the brain. It can be concluded that curcumin has some affinity towards some tissues. It was stated that the metabolism of curcumin depends on the route of administration, it was found that for oral administration 75% of curcumin metabolites were found in feces and not in urine (Holder et al. 1979). On the other hand, when the curcumin was given intraperitoneally, 73% of curcumin metabolites were found in urine and only 11% was absorbed in body. It was observed from human and mouse trials that oral consumption of curcumin showed lesser bioavailability, and it undergoes intestinal metabolism, whereas the absorbed curcumin have rapid metabolism and excretion in bile (Saikia et al. 2015).

Hence, the major limitations of curcumin include low absorption, poor solubility and relatively less distribution property. Hence, curcumin is not absorbed properly in the body and cannot be utilized for the treatment of diseases (Rai et al. 2015, Saikia et al. 2015). Curcumin possesses low bioavailability due to rapid metabolism, rapid elimination, poor distribution in tissue and low serum level (Saikia et al. 2015). Curcumin has many limitations but the bioavailability of curcumin can be improved by suitable encapsulation of curcumin. For example, if curcumin is administered by encapsulating in liposome, polymeric nanoparticles, cyclodextrin and other lipid or polymer curcumin complexes it can improve the biological activity of curcumin (Nanjwade et al. 2015).

Conclusions

Curcuma longa (turmeric) is one of the most important Indian spices, which is being used traditionally since ancient times as a spice and potent medicine. The validation of turmeric proved that it contains a yellow pigment, curcumin, which has enormous medicinal properties such as antimicrobial activity, anti-inflammatory activity, wound healing activity, anticancerous activity, antioxidant activity, anti-arthritis, antimalarial, chemopreventive and chemotherapeutic activity and many more. Hence, it is considered a safe, nontoxic and effective herbal medicine for a variety of infections and diseases including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, liver disorders, Alzheimer's, Parkinson's and small-pox disease, etc. In addition, its specific role in the treatment of different types of cancer is very promising. However, further studies are required to determine the optimal dosage, bioavailability and bio-efficacy, which may help in popularization of curcumin for its use as one of the ideal herbal medicines.

References

Aboueldahab, M.M. and Elhussieny, E.A. 2016. Antiparasitic and physiological evaluation of *Curcuma longa* extract and/or PZQ on *Schistosoma mansoni* infected mice. International Int. J. Adv. Res. 4(6): 1020–1039.

Aggarwal, B.B. and Harikumar, K.B. 2009. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int. J. Biochem. Cell B. 41: 40–59.

Ahmed, T. and Gilani, A.H. 2009. Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine induced amnesia may explain medicinal use of turmeric in Alzheimer's disease. Pharmacol. Biochem. Be. 91: 554–559.

Ahmed, T. and Gilani, A.H. 2014. Therapeutic Potential of Turmeric in Alzheimer's Disease: Curcumin or Curcuminoids? Phytother. Res. 28: 517–525.

Akbik, D., Ghadiri, M., Chrzanowski, W. and Rohanizadeh, R. 2014. Curcumin as a wound healing agent. Life Sci. 116(1): 1–7.

Aoki, H., Moro, O., Tagami, H. and Kishimoto, J. 2007. Gene expression profiling analysis of solar lentigo in relation to immunohistochemical characteristics. Br. J. Dermatol. 156: 1214–1223.

Arca, E., Tastan, H.B., Erbil, A.H., Sezer, E., Koc, E. and Kurumlu, Z. 2006. Narrow-band ultraviolet B as monotherapy and in combination with topical calcipotriol in the treatment of vitiligo. J. Dermatol. 33(5): 338–343.

Asawanonda, P. and Klahan, S.O. 2010. Tetrahydrocurcuminoid cream plus targeted narrowband UVB phototherapy for vitiligo: a preliminary randomized controlled study. Photomed. Laser Surg. 28(5): 679–684.

Asghari, G., Mostajeran, A. and Shebli, M. 2009. Curcuminoid and essential oil components of turmeric at different stages of growth cultivated in Iran. Res. Pharmac. Sci. 4(1): 55–61.

Basnet, P. and Skalko-Basnet, N. 2011. Curcumin: An anti-inflammatory molecule from a curry spice on the path to cancer treatment. Molecules 16: 4567–4598.

Baum, L., Lam, C.W., Cheung, S.K., Kwok, T., Lui, V., Tsoh, J., Lam, L., Leung, V., Hui, E., Ng, C., Woo, J., Chiu, H.F., Goggins, W.B., Zee, B.C., Cheng, K.F., Fong, C.Y., Wong, A., Mok, H., Chow, M.S., Ho, P.C., Ip, S.P., Ho, C.S., Yu, X.W., Lai, C.Y., Chan, M.H., Szeto, S., Chan, I.H. and Mok, V. 2008. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer Disease. J. Clin. Psychopharmacol. 28(1): 110–3.

Begum, A.N., Jones, M.R., Lim, G.P., Morihara, T., Kim, P., Heath, D.D., Rock, C.L., Pruitt, M.A., Yang, F., Hudspeth, B., Hu, S., Faull, K.F., Teter, B., Cole and Frautschy, S.A. 2008. Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's Disease. J. Pharmacol. Exp. Ther. 326: 196–208.

Blakytny, R. and Jude, E. 2006. The molecular biology of chronic wounds and delayed healing in diabetes. Diabet. Med. 23: 594–608.

Blennow, K., de Leon, M.J. and Zetterberg, H. 2006. Alzheimer's disease. Lancet. 368: 387–403.

Bosman, B. 1994. Testing of lipoxygenase inhibitors, cyclooxygenase inhibitors, drugs with immunomodulating properties and some reference antipsoriatic drugs in the modified mouse tail test, an animal model of psoriasis. Skin Pharmacol. 7(6): 324–334.

Chainani-Wu, N. 2004. Safety and anti-inflammatory activity of curcumin: a component of tumeric (*Curcuma longa*). J. Altern. Complem. Med. 9(1): 161–168.

Chakrabarti, R., Rawat, P.S., Cooke, B.M., Coppel, R.L. and Patankar, S. 2013. Cellular effects of curcumin on plasmodium falciparum include disruption of microtubules. PLoS ONE 8(3): 1–14.

Chandran, B. and Goel, A. 2012. A randomized, pilot study to assess the efficacy and safety of curcumin in patients with active rheumatoid arthritis. Phytother. Res. 26: 1719–1725.

Chatterjee, S., Datta, R.N., Bhattacharyya, D. and Bandhopadhyay, S.K. 2005. Emollient and antipruritic effect of Itch cream in dermatological disorders: A randomized controlled trial. Indian J. Pharmaco. 37(4): 253–254.

Chen, D.Y., Shien, J.H., Tiley, L., Chiou, S.S.,Wang, S.Y., Chang, T.J., Lee, Y.J., Chan, K.W. and Hsu, W.L. 2010. Curcumin inhibits influenza virus infection and haemagglutination activity. Food Chem. 119(1): 1346–1351.

Cheng, A.L., Hsu, C.H., Lin, J.K., Hsu, M.M., Ho, Y.F., Shen, T.S., Ko, J.Y., Lin, J.T., Lin, B.R., Ming-Shiang, W., Yu, H.S., Jee, H.S., Chen, G.S., Chen, T.M., Chen, C.A., Lai, M.K., Pu, Y.S., Pan, M.H., Wang, Y.J.,Tsai, C.C. and Hsieh, C.Y. 2001. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. Anticancer Res. 21(4B): 2895–2900.

Cheng, K.K., Yeung, C.F., Ho, S.W., Chow, S.F., Chow, A.H.L. and Baum, L. 2013. Highly stabilized curcumin nanoparticles tested in an *in vitro* blood–brain barrier model and in Alzheimer's Disease Tg2576 Mice. The AAPS Journal. 15(2): 324–336.

Chong, L., Zhang, W., Nie, Y., Yu, G., Liu, L., Lin, L., Wen, S., Zhu, L. and Li, C. 2014. Protective effect of curcumin on acute airway inflammation of allergic asthma in mice through Notch1-GATA3 signaling pathway. Inflammation 37: 1476–1485.

Cleaver, J.E. 1968. Defective repair replication of DNA in xeroderma pigmentosum. Nature 218: 652–656.

Daily, J.W., Yang, M. and Park, S. 2016. Efficacy of turmeric extracts and curcumin for alleviating the symptoms of joint arthritis: a systematic review and meta-analysis of randomized clinical trials. J. Med. Food 19(8): 717–729.

Dauer, W. and Przedborski, S. 2003. Parkinson's disease mechanisms and models. Neuron. 39: 889–909.

Davidson, S. and Giesler, G.J. 2010. The multiple pathways for itch and their interactions with pain. Trends Neurosci. 33: 550–558.

Dawson, T.M. and Dawson, V.L. 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science 302: 819–822.

De Clercq, E. 2000. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med. Res. Rev. 20(1): 323–349.

Dyab, A.K., Yones, D.A., Ibraheim, Z.Z. and Hassan, T.M. 2016. Anti-giardial therapeutic potential of dichloromethane extracts of *Zingiber officinale* and *Curcuma longa in vitro* and *in vivo*. Parasitol. Res. 115(1): 2637–2645.

Edwards, P.C. and Kelsch, R. 2002. Oral lichen planus: clinical presentation and management. J. Can. Dent. Assoc. 68(8): 494–499.

El-Habibi, E.S.M., El-Wakf, A.M. and Mogall, A. 2013. Efficacy of curcumin in reducing risk of cardiovascular disease in high fat diet-fed rats. J. Bioanal. Biomed. 5(1): 066–070.

Emiroglu, G., Coskun, Z.O., Kalkan, Y., Erdivanli, O.C., Tumkaya, L., Terzi, S., Özgür, A., Demirci, M. and Dursun, E. 2017. "The Effects of Curcumin on Wound Healing in a Rat Model of Nasal Mucosal Trauma," Evid.-Based Compl. Alt., vol. 2017, Article ID 9452392, 6 pages. doi:10.1155/2017/9452392.

Ferreira, V.H., Nazli, A., Dizzell, S.E., Mueller, K. and Kaushic, C. 2015. The anti-inflammatory activity of curcumin protects the genital mucosal epithelial barrier from disruption and blocks replication of HIV-1 and HSV-2. PLoS ONE 10(4): e0124903. https://doi.org/10.1371/journal.pone.0124903.

Filiberto, G.G., Lissethe, P.L., José, M.H., Armando, P.R., Rodrigo, A.O., Alicia, H.C., Rafael, C., Flores, D.J., Lee, L.H. and Adams, S.D. 2016. Inhibition of curcumin-treated herpes simplex virus 1 and 2 in vero cells. Adv. Microbiol. 6(1): 276–287.

Fouladvand, M., Barazesh, A. and Tahmasebi, R. 2013. Evaluation of *in vitro* antileishmanial activity of curcumin and its derivatives "gallium curcumin, indium curcumin and diacethyle curcumin". Eur. Rev. Med. Pharmaco. 17(1): 3306–3308.

Fujisawa, S., Atsumi, T., Ishihara, M. and Kadoma, Y. 2004. Activity of curcumin and related compounds. Anticancer Res. 24: 563–570.

Ghiso, J. and Frangione, B. 2002. Amyloidosis and Alzheimer's Disease. Adv. Drug Deliver. Rev. 54: 1539–1551. doi:10.1016/S0169-409X(02)00149-7.

Goel, A., Kunnumakkara, A.B. and Aggarwal, B.B. 2008. Curcumin as ''curecumin'': from kitchen to clinic. Biochem. Pharmacol. 2008. 75: 787–809.

Gupta, S.C., Patchva, S. and Aggarwal, B.B. 2012. Therapeutic roles of curcumin: Lessons learned from clinical trials. AAPS J. 15(1): 195–218.

Gutiérrez, J.M. and Lomonte, B. 1995. Phospholipase A2 myotoxins from *Bothrops* snake venoms. Toxicon. 33(11): 1405–1424.

Haldeman, G.A., Croft, J.B., Giles, W.H. and Rashidee, A. 1999. Hospitalization of patients with heart failure: national hospital discharge survey, 1985 to 1995. Am. Heart J. 137(2): 352–360.

Hanai, H., Lida, T., Takeuchi, K., Watanabe, F., Maruyama, Y., Andoh, A., Tsujikawa, T., Fujiyama, Y., Mitsuyama, K., Sata, M., Yamada, M., Iwaoka, Y., Kanke, K., Hiraishi, H., Hirayama, K., Aria, H., Yoshii, S., Uchijima, M., Nagata, T. and Koide, Y. 2006. Curcumin maintenance therapy for ulcerative colitis: randomized, multicentre, double-blind, placebo-controlled trial. Clin. Gastroeterol. Hepatol. 4(12): 1502–1506.

Hanselmann, C., Mauch, C. and Werner, S. 2001. Haem oxygenase-1: a novel player in cutaneous wound repair and psoriasis? Biochem. J. 353(3): 459–466.

Hardy, J. and Selkoe, D.J. 2002. The amyloid hypothesis of Alzheimer's Disease: Progress and problems on the road to therapeutics. Science 297: 353–356. doi:10.1126/science.1072994.

He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S. and Du, Z. 2015. Curcumin, inflammation, and chronic diseases: how are they linked? Molecules 20(5): 9183–9213.

Heng, M.C., Song, M.K. and Heng, M.K. 1994. Elevated phosphorylase kinase activity in psoriatic epidermis: Correlation with increased phosphorylation and psoriatic activity. Br. J. Dermatol. 130(3): 298–306.

Heng, M.C., Song, M.K., Harker, J. and Heng, M.K. 2000. Drug-induced suppression of phosphorylase kinase activity correlates with resolution of psoriasis as assessed by clinical, histological and immunohistochemical parameters. Br. J. Dermatol. 143(5): 937–949.

Hermann, P.T. and Ammo Wahl, M.A. 1991. Pharmacology of *Curcuma longa.* Planta Med. 57: 1–7.

Hilsden, R.J., Verhoef, M.J., Rasmussen, H., Porcino, A. and DeBruyn, J.C. 2011. Use of complementary and alternative medicine by patients with inflammatory bowel disease. Inflamm. Bowel. Dis. 17(1): 655–662.

Holder, G.M., Plummer, J.L. and Ryan, A.J. 1978. The metabolism and excretion of curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) in the rat. Xenobiotica 8: 761–768.

Holt, P.R., Katz, S. and Kirshoff, R. 2005. Curcumin therapy in inflammatory bowel disease: a pilot study. Dig. Dis. Sci. 50(1): 2191–2193.

Iwata, N., Higuchi, M. and Saido, T.C. 2005. Metabolism of amyloid-β peptide and Alzheimer's disease. Pharmacol. Therapeut. 108: 129–148. doi:10.1016/j.pharmthera.2005.03.010.

Jagetia, G. and Rajanikant, G. 2012. Acceleration of wound repair by curcumin in the excision wound of mice exposed to different doses of fractionated gamma radiation. Int. Wound J. 9: 76–92.

Jain, A. and Basal, E. 2003. Inhibition of *Propionibacterium acnes*-induced mediators of inflammation by Indian herbs. Phytomedicine 10(1): 34–38.

Jana, S. and Swarnakar, S. 2013. Cardiovascular diseases: nutritional and therapeutic interventions, publisher group; Taylor and Francis Group, Editors; Nilanjana Maulik 1(26): 487–500.

Jankovic, J. 2008. Parkinson's disease: clinical features and diagnosis. J. Neurol. Neurosur. Ps. 79: 368–76.

Javvadi, P., Segan, A.T., Tuttle, S.W. and Koumenis, C. 2008. The Chemopreventive agent curcumin is a potent radiosensitizer of human cervical tumor cells via increased reactive oxygen species production and overactivation of the mitogen-activated protein kinase Pathway. Mol. Pharmacol. 73(5): 1491–1501. doi:10.1124/mol.107.043554.

Jiang, A.J., Jiang, G., Li, L.T. and Zheng, J.N. 2015. Curcumin induces apoptosis through mitochondrial pathway and caspases activation in human melanoma cells. Mol. Biol. Rep. 42: 267–275. doi:10.1007/s11033-014-3769-2.

Jiao, Y., Wilkinson, J.I.V., Christine Pietsch, E., Buss, J.L., Wang, W., Planalp, R., Torti, F.M. and Torti, S.V. 2006. Iron chelation in the biological activity of curcumin. Free Radical Biol. Med. 40: 1152–60.

Jiao, Y., Wilkinson, IV.J., Di, X., Wang, W., Hatcher, H., Kock, N.D., D'Agostino, R., Knovich, Jr. M.A., Torti, F.M. and Torti, S.V. 2009. Curcumin, a cancer chemopreventive and chemotherapeutic agent, is a biologically active iron chelator. Blood. 113(2): 462–469. https://dx.doi.org/10.1182%2Fblood-2008-05-155952.

Joe, B., Vijaykumar, M. and Lokesh, B. 2004. Biological properties of curcumin-cellular and molecular mechanisms of action. Crit. Rev. Food Sci. Nutr. 44: 97–111.
Johnson, V.L. and Hunter, D.J. 2014. The epidemiology of osteoarthritis. Best Pract. Res. Clin. Rheumatol. 28(1): 5–15.
Kadekaro, A.L., Kavanagh, R.J., Wakamatsu, K., Ito, S., Pipitone, M.A. and Abdel-Malek, Z.A. 2003. Cutaneous photobiology. The melanocyte vs. the sun: who will win the final round? Pigment. Cell Res. 16: 434–447.
Kali, A., Bhuvaneshwar, D., Charles, P.M. and Seetha, K.S. 2016. Antibacterial synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates. J. Basic Clin. Pharma. 7(1): 93–96.
Kanlayavattanakul, M. and Lourith, N. 2011. Therapeutic agents and herbs in topical application for acne treatment. Int. J. Cosmet. Sci. 33: 289–97.
Karunagaran, D., Rashmi, R. and Kumar, T.R. 2005. Induction of apoptosis by curcumin and its implications for cancer therapy. Curr. Cancer Drug Targets. 5(2): 117–29.
Kastan, M.B. and Bartek, J. 2004. Cell-cycle checkpoints and cancer. Nature 432(7015): 316–23, ISSN 1476–4687.
Kastelan, L.M., Massari, P. and Brajac, I. 2006. Apoptosis mediated by cytolytic molecules might be responsible for maintenance of psoriatic plaques. Med. Hypotheses 67(2): 336–337.
Khiljee, S., Rehman, N.U., Khiljee, T., Saeed, A.R., Khan, M.Y. and Qureshi, U.A. 2011. Use of traditional herbal medicines in the treatment of eczema. J. Pak. Assoc. Dermatol. 21: 112–117.
Kim, S.J., Son, T.G., Park, H.R., Park, M., Kim, M.S., Kim, H.S., Chung, H.Y., Mattson, M.P. and Lee, J. 2008. Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. J. Biol. Chem. 283: 14497–505.
Kojima, H., Yanai, T. and Toyota, A. 1998. Essential oil constituents from Japanese and Indian *Curcuma aromatica* rhizomes. Planta Med. 64: 380–381.
Kulac, M., Aktas, C., Tulubas, F., Uygur, R., Kanter, M., Erboga, M., Ceber, M., Topcu, B. and Ozen, O.A. 2013. The effects of topical treatment with curcumin on burn wound healing in rats. J. Mol. Histol. 44(1): 83–90.
Kumar, K. and Rai, A. 2011. Curcumin: a yellow magical spice of kitchen for treatment of rheumatoid arthritis. Int. Res. J. Pharm. 2(12): 29–31.
Kundu, P., Das, M., Tripathy, K. and Sahoo, S.K. 2016. Delivery of dual drug loaded lipid based nanoparticles across the blood−brain barrier impart enhanced neuroprotection in a rotenone induced mouse model of Parkinson's Disease. ACS Chem. Neurosci. 7: 1658−1670.
Kurup, V.P. and Barrios, C.S. 2008. Immunomodulatory effects of curcumin in allergy. Mol. Nutr. Food Res. 52: 1031–1039.
Lalla, J.K., Nandedkar, S.Y., Paranjape, M.H. and Talreja, N.B. 2001. Clinical trials of ayurvedic formulations in the treatment of acne vulgaris. J. Ethnopharmacol. 78: 99–102.
Lebwohl, M. 2004. Innovations in the treatment of psoriasis. J. Am. Acad. Dermatol. 51(1): 40–41.
Lee, W.H., Loo, C.Y., Bebawy, M., Luk, F., Mason, R.S. and Rohanizadeh R. 2013. Curcumin and its derivatives: Their application in neuropharmacology and neuroscience in the 21st century. Curr. Neuropharmacol. 11: 338–378.
Lim, G.P., Chu, T., Yang, F., Beech, W., Frautschy, S.A. and Cole, G.M. 2001. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J. Neurosci. 21: 8370–7.
Lin, M.S., Hung, K.S., Chiu, W.T., Sun, Y.Y., Tsai, S.H., Lin, J.W. and Lee, Y.H. 2011. Curcumin enhances neuronal survival in N-methyl-d-aspartic acid toxicity by inducing RANTES expression in astrocytes *via* PI-3K and MAPK signaling pathways. Prog. Neuro-Psychoph. 35: 931–8.
Lodi, G., Scully, C., Carrozzo, M., Griffiths, M., Sugerman, P.B. and Thongprasom, K. 2005. Current controversies in oral lichen planus: Report of an international consensus meeting. Part 2. Clinical management and malignant transformation. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. 100(2): 164–178.
Loughlin, D. and Artlett, C. 2004. Modification of collagen by 3-deoxyglucosone alters wound healing through differential regulation of p38 MAP kinase. PLoS One 6(5): 18676.
Lu, J.J., YeDang, Y., Huang, M., Xu, W.S., Chen, X.P. and Wang, Y.T. 2012. Anti-cancer properties of terpenoids isolated from Rhizoma Curcumae—a review. J. Ethnopharmacol. 143: 406–411.
Ma, F., Liu, F., Ding, L., Ming, Y., Yue, H., Zhou, Y. and Hou, Y. 2017. Anti-inflammatory effects of curcumin are associated with down regulating microRNA-155 in LPS-treated macrophages and mice. Pharm. Biol. 55(1): 1263–1273. DOI:10 .1080/13880209.2017.1297838.
Mandroli, P.S. and Bhat, K. 2013. An *in vitro* evaluation of antibacterial activity of curcumin against common endodontic bacteria. J. Appl. Pharm. Sci. 3(10): 106–108.
Martins, C.V.B., daSilva, D.S., Neres, A.T.M., Magalha, T.F.F., Watanabe, G.A., Modolo, L.V., Sabino, A.A., deFátima, A. and deResende M.A. 2009. Curcumin as a promising antifungal of clinical interest. J. Antimicrob. Chemoth. 63(1): 337–339.
Mathew, A., Yoshida, Y., Maekawa, T. and Sakthi Kumar, D. 2011. Alzheimer's disease: Cholesterol a menace? Brain Res. Bull. 86: 1–12. doi:10.1016/j.brainresbull.2011.06.006.
Mathew, A., Aravind, A., Brahatheeswaran, D., Fukuda, T., Nagaoka, Y., Hasumura, T., Iwai, S., Morimoto, H., Yoshida, Y., Maekawa, T., Venugopal, K. and Kumar, D.S. 2012. Amyloid-binding aptamer conjugated curcumin–PLGA nanoparticle for potential use in Alzheimer's Disease. Bionanosci 2: 83–93. DOI:10.1007/s12668-012-0040-y.
Mawuenyega, K.G., Sigurdson, W., Ovod, V., Munsell, L., Kasten, T., Morris, J.C., Yarasheski, K.E. and Bateman, R.J. 2010. Decreased clearance of CNS β-amyloid in Alzheimer's disease. Science 330(6012): 1774. doi:10.1126/science.1197623.
Mazumdar, A., Raghavan, K., Weinstein, J., Kohn, K.W. and Pommer, Y. 1995. Inhibition of human immunodeficiency virus type-1 integrase by curcumin. Biochem. Pharmacol. 49(1): 1165–1170.

Meng, B., Li, J. and Cao, H. 2013. Antioxidant and antiinflammatory activities of curcumin on diabetes mellitus and its complications. Curr. Pharm. 19: 2101–13.

Menon,V.P. and Sudheer A.R. 2007. Antioxidant and anti-inflammatory properties of curcumin. Adv. Exp. Med. Biol. 595: 105–125.

Micucci, M., Aldini, R., Cevenini, M., Colliva, C., Spinozzi, S., Roda, G., Montagnani, M., Camborata, C., Camarda, L., Chiarini, A., Mazzella, G. and Budriesi, R. 2013. *Curcuma longa* L. as a therapeutic agent in intestinal motility disorders. 2: safety profile in mouse. PLoS ONE 8(11): 1–13.

Miguel, C.G., Ana, R.B., Vicente, N.L., Asunción, M.A., Manuel, A.L., August, B. and José, H.F. 2015. Effects of curcuma extract and visible light on adults with plaque psoriasis. Eur. J. Dermatol. 25(3): 240–246.

Miquel, A.J., Bernd, J.M., Sempere, J., Diaz, A. and Ramirez, A. 2002. The curcuma antioxidants: Pharmacological effects and prospects for future clinical use. A review. Arch. Gerontol. Geriatr. 34(1): 37–46.

Mirzabeigi, P., Mohammadpour, A.H., Salarifar, M., Gholami, K., Mojtahedzadeh, M. and Javadi, M.R. 2015. The effect of curcumin on some of traditional and non-traditional cardiovascular risk factors: a pilot randomized, double-blind, placebo-controlled trial. Iran. J. Pharm. Res. 14(1): 479–486.

Mohanty, C., Das, M. and Sahoo, S. 2012. Sustained wound healing activity of curcumin loaded oleic acid based polymeric bandage in a rat model. Mol. Pharm. 9: 2801–11.

Mohanty, C. and Sahoo, S.K. 2017. Curcumin and its topical formulations for wound healing applications. Drug Discov. Today. S1359-6446(16): 30437–8. doi: 10.1016/j.drudis.2017.07.001.

Morais, E.R., Oliveira, K.C., Magalhães, L.G., Moreira, E.B., Sergio, V.A. and Rodrigues, V. 2013. Effects of curcumin on the parasite *Schistosoma mansoni*: A transcriptomic approach. Mol. Biochem. Parasit. 187(1): 91–97.

Mun, S.H., Joung, D.K., Kim, Y.S., Kang, O.H., Kim, S.B., Seo, Y.S., Kim, Y.C., Lee, D.S., Shin, D.W., Kweon, K.T. and Kwon, D.Y. 2013. Synergistic antibacterial effect of curcumin againstmethicillin-resistant *Staphylococcus aureus*. Phytomedicine 20(8-9): 714–718.

Muthane, U., Yasha, T.C. and Shankar, S.K. 1998. Low numbers and no loss of melanized nigral neurons with increasing age in normal human brains from India. Ann. Neurol. 43: 283–7.

Mythri, R.B., Jagatha, B., Pradhan, N., Andersen, J. and Bharath, M.M.S. 2007. Mitochondrial complex I inhibition in Parkinson's disease: how can curcumin protect mitochondria? Antioxid. Redox Sign. 9(3): 399–408. doi: 10.1089/ars.2006.1479.

Mythri, R.B., Harish, G., Dubey, S.K, Misra, K. and Bharath, M.M.S. 2011. Glutamoyl diester of the dietary polyphenol curcumin offers improved protection against peroxynitrite-mediated nitrosative stress and damage of brain mitochondria *in vitro*: implications for Parkinson's disease. Mol. Cell. Biochem. 347: 135–143. DOI:10.1007/s11010-010-0621-4.

Nadkarni, K.M. 1976. Indian Materia Medica 1.3rd ed. Bombay: Popular Prakasan.

Nanjwade, B.K., Bellad, K.A., Mohamied, A.S., Nwaji, M.S. and Srichana, T. 2015. Curcumin: nutraceutical and pharmaceutical applications. Adv. Pharmacog. Phytomed. 1(1): 17–26.

Neto, Z., Machado, M., Lindeza, A., Rosário, V.D., Gazarini, M.L. and Lopes, D. 2013. Treatment of *Plasmodium chabaudi* parasites with curcumin in combination with antimalarial drugs: drug interactions and implications on the ubiquitin/proteasome system. J. Parasitol. Res. (1): 1–11.

Nilani, P., Kasthuribai, N., Duraisamy, B., Dhamodaran, P., Ravichandran, S., Ilango, K. and Suresh, B. 2009. *In vitro* antioxidant activity of selected antiasthmatic herbal constituents. Anc. Sci. Life 28: 3–6.

Nirmal, N., Praba, G.O. and Velmurugan, D. 2008. Modeling studies on phospholipase A2-inhibitor complexes. Indian Journal of Biochemistry and Biophysic. 45(4): 256–262.

Novaes, R.D., Sartini, M.V.P., Rodriguesa, J.P.F., Gonçalves, R.V., Santos, E.C., Souza, R.L.M. and Caldas, I.S. 2016. Curcumin enhances the anti-*Trypanosoma Cruzi* activity of benznidazole-based chemotherapy in acute experimental chagas disease. Antimicrob. Agents Chemother. 60(6): 3355–64.

Ownby, C.L. 1998. Structure, function and biophysical aspects of the myotoxins from snake venoms. J. Toxicol Toxins Rev. 17(1): 213–238.

Padmanaban, G., Nagaraj, V.A. and Rangarajan, P.N. 2012. Artemisinin-based combination with curcumin adds a new dimension to malaria therap. Curr. Sci. 102(5): 704–711.

Pal, R., Miranda, M. and Narayan, M. 2011. Nitrosative stress-induced parkinsonian lewy-like aggregates prevented through polyphenolic phytochemical analog intervention. Biochem. Bioph. Res. Co. 404: 324–9.

Pan, M.H., Huang, T.M. and Lin, J.K. 1998. Biotransformation of curcumin through reduction and glucuronidation in mice. Drug Metab. Dispos. 27: 486–494. doi:0090-9556/99/2704-0486-0494.

Pandey, N., Strider, J., Nolan, W.C., Yan, S.X. and Galvin, J.E. 2008. Curcumin inhibits aggregation of alpha-synuclein. Acta Neuropathol. 115: 479–89.

Paul, S.C., Krishnakumar, K. and Kavitha M.P. 2016. Phyto-Pharmacology of Curcuma longa: A Review. Int. J. Pharmacol. Res. 6: 144–147.

Pol, A., Bergers, M. and Schalkwijk, J. 2003. Comparison of antiproliferative effects of experimental and established antipsoriatic drugs on human keratinocytes, using a simple 96-well-plate assay. *In Vitro* Cell Dev. Biol. Anim. 39(1-2): 36–42.

Pongrakhananon, V. and Rojanasakul, Y. 2011. Anticancer Properties of Curcumin. "Advances in Cancer Therapy", book edited by HalaGali-Muhtasib, ISBN 978-953-307-703-1, pp. 347–368.

Pulido-Moran, M., Moreno-Fernandez, J., Ramirez-Tortosa, C. and Ramirez-Tortosa, M. 2016. Curcumin and health. Molecules 21(3): 264. doi: 10.3390/molecules21030264.

Qian, Y., Ma, J. and Guo X. 2015. Curcumin enhances the radiosensitivity of U87 cells by inducing DUSP-2 up-regulation. Cell Physiol. Biochem. 35: 1381–1393.
Rahman, I. and Biswas, S.K. 2009. Regulation of inflammation, redox, and glucocorticoid signaling by dietary polyphenols. *In*: Surh, Y.J., Dong, Z., Cadenas, E. and Packer, L. (eds.). Boca Raton: CRC Press.
Rai, M., Pandit, R., Gaikwad, S., Yadav, A. and Gade, A. 2015. Potential applications of curcumin and curcumin nanoparticles: from traditional therapeutics to modern nanomedicine. Nanotechnol. Rev. 4(2): 161–172.
Rajeswari, A. and Sabesan, M. 2008. Inhibition of monoamine oxidase-B by the polyphenolic compound, curcumin and its metabolite tetrahydrocurcumin, in a model of Parkinson's disease induced by MPTP neurodegeneration in mice. Inflammopharmacol 16: 96–9.
Raza, H., John, A., Brown, E.M., Benedict, S. and Kambal, A. 2008. Alterations in mitochondrial respiratory functions, redox metabolism and apoptosis by oxidant 4-hydroxynonenal and antioxidants curcumin and melatonin in PC12 cells. Toxicol. Appl. Pharmacol. 226: 161–8.
Ringman, J.M., Frautschy, S.A., Cole, G.M., Masterman, D.L. and Cummings, J.L. 2005. A potential role of the curry spice curcumin in Alzheimer's Disease. Curr. Alzheimer Res. 2(2): 131–6.
Ryu, E.K., Choe, Y.S., Lee K.H., Choi, Y. and Kim, B.T. 2006. Curcumin and dehydrozingerone derivatives: Synthesis, radiolabeling, and evaluation for beta-amyloid plaque imaging. J. Med. Chem. 49: 6111–6119.
Sadhana, S. and Gupta, A.K. 2013. Evaluation of phenolics content, flavonoids and antioxidant activity of *Curcuma amada* (Mango Ginger) and *Zingiber officinale* (Ginger). Res. Rev. J. Chem. 2(1): 32–35.
Saikia, C., Sarmah, M., Das, M.K., Ramteke, A. and Maji, T.K. 2015. Biodegradable Polymeric Nanocomposites: Advances in Biomedical Applications. Chap. 8 Taylor and Francis Publication Boca Raton.
Samy, R.P., Gopalakrishnakone, P. and Chow, V.T. 2012. Therapeutic application of natural inhibitors agaisnt snake venom phospholipase A2. Bioinformation 8(1): 048–057.
Santos, A.M., Lopes, T., Oleastro, M., Gato, I.V., Floch, P., Benejat, L., Chaves, P., Pereira, T., Seixas, E., Machado, J. and Guerreiro, A.S. 2015. Curcumin inhibits gastric inflammation induced by *Helicobacter pylori* infection in a mouse model. Nutrients 7(1): 306–320.
Sasidharan, N.K., Sreekala, S.R., Jacob, J. and Nambisan, B. 2014. *In vitro* synergistic effect of curcumin in combination with third generation cephalosporins against bacteria associated with infectious diarrhea. Biomed. Res. Int. (1): 1–8.
Satoh, Y. 1992. Japanese Skin Type (JST). Proc. Jap. Comm. for Sunlight Prot. 2: 62–70.
Schallreuter, K.U., Wood, J.M., Pittelkow, M.R., Buttner, G., Swanson, N. and Korner, C. 1996. Increased monoamine oxidase A activity in the epidermis of patients with vitiligo. Arch. Dermatol. Res. 288(1): 14–18.
Sharma, M., Manoharlal, R., Puri, N. and Prasad, R. 2010. Antifungal curcumin induces reactive oxygen species and triggers an early apoptosis but prevents hyphae development by targeting the global repressor *TUP1* in *Candida albicans*. Biosci. Rep. 30(1): 391–404.
Shanmugam, M.K., Rane, G., Kanchi , M.M., Arfuso, F., Chinnathambi , A., Zayed , M.E., Alharbi , S.A., Tan , B.K.H., Kumar, A.P. and Sethi, G. 2015. The multifaceted role of curcumin in cancer prevention and treatment. Molecules, 2728–2769.
Shishodia, S., Sethi, G. and Aggarwal, B.B. 2005. Curcumin: getting back to the roots. Ann. NY Acad. Sci. 1056(1): 206–217.
Shrishail, D.H., Harish, K., Ravichandra, H., Tulsianand, G. and Shruthi, S.D. 2013. Turmeric: Nature's precious medicine. Asian J. Pharm. Clin. Res. 6(3): 10–16.
Signaling by Dietary Polyphenols. *In*: Surh, Y.J., Dong, Z., Cadenas, E. and Packer, L. (eds.). Boca.
Singh, R.P. and Jain, D.A. 2012. Evaluation of antimicrobial activity of curcuminoids isolated from turmeric. Int. J. Pharm. Life Sci. 3(1): 1368–1376.
Singh, V., Pal, M., Gupta, S., Tiwari, S.K., Malkunje, L. and Das, S. 2013. Turmeric—a new treatment option for lichen planus: a pilot study. Natl. J. Maxillofac. Surg. 4: 198–201.
Sirenia, G.P., Rafael, C.Z., Mario, A.R., María, L.M. and Araceli, C.R. 2017. Curcumin alters the cytoskeleton and microtubule organization on trophozoites of *Giardia lamblia*. Acta Tropica. 172(1): 113–121.
Sofic, E., Riederer, P., Heinsen, H., Beckmann, H., Reynolds, G.P., Hebenstreit, G. and Youdim, M.B.H. 1988. Increased iron (III) and total iron content in post mortem *substantia nigra* of parkinsonian brain. J. Neural Transm. 74: 199–205.
Sommerfeld, B. 2007. Randomised, placebo-controlled, double-blind, split-face study on the clinical efficacy of Tricutan® on skin firmness. Phytomedicine 14(11): 711–715.
Sun, M., Gao, Y., Guo, C., Cao, F., Song, Z., Xi, Y., Yu, A., Li, A. and Zhai, G. 2010. Enhancement of transport of curcumin to brain in mice by poly(n-butylcyanoacrylate) nanoparticle. J. Nanopart. Res. 12: 3111–3122.
Szczepanowicz, K., Jantas, D., Piotrowski, M., Staroń, J., Leśkiewicz, K., Regulska, M., Lasoń, W. and Warszyński, P. 2016. Encapsulation of curcumin in polyelectrolyte nanocapsules and their neuroprotective activity. Nanotechnology 27: 355101.
Taher, M.M., Lammering, G., Hershey, C. and Valerie, K. 2003. Curcumin inhibits ultraviolet light induced human immunodeficiency virus gene expression. Mol. Cell Biochem. 254(1): 289–297.
Takahashi, Y., Fukushima, Y., Kondo, K. and Ichihashi, M. 2017. Facial skin photo-aging and development of hyperpigmented spots from children to middle-aged Japanese woman. Skin Res. Technol. 23(4): 613–618.
Taylor, J.S. 2002. New structural and mechanistic insight into the A-rule and the instructional and non-instructional behavior of DNA photoproducts and other lesions. Mutat. Res. 510: 55–70.
Teixeira, C., Cury, Y., Moreira, V., Picolob, G. and Chaves, F. 2009. Inflammation induced by *Bothrops asper* venom. Toxicon. 54(1): 988–997.
Thangapazham, R., Sharad, S. and Maheshwari, R. 2013. Skin regenerative potentials of curcumin. Biofactors 39: 141–9.

Thangapazham, R.L., Sharma, A. and Maheshwari, R.K. 2007. Beneficial role of curcumin in skin diseases. *In*: Aggarwal, B.B., Surh, Y.J. and Shishodia, S. (eds.). Boston: Springer.

Tonnesen, H.H., deVries, H., Karlsen, J. and Henegouwen, G.B.V. 1987. Studies on curcumin and curcuminoids. IX: Investigation of the photobiological activity of curcumin using bacterial indicator systems. J. Pharm Sci. 76(5): 371–373.

Tyagi, P., Singh, M., Kumari, H., Kumari, A. and Mukhopadhyay, K. 2015. Bactericidal activity of curcumin I is associated with damaging of bacterial Membrane. PLoS ONE 10(3): 1–15.

Ullah, F., Liang, A., Rangel, A., Gyengesi, E., Niedermayer, G. and Munch, G. 2017. High bioavailability curcumin: an anti-inflammatory and neurosupportive bioactive nutrient for neurodegenerative diseases characterized by chronic neurooinflammation. Arch. Toxicol. 91(4): 1623–1634. https://doi.org/10.1007/s00204-017-1939-4.

Vajragupta, O., Boonchoong, P., Watanabe, H., Tohda, M., Kummasud, N. and Sumanont, Y. 2003. Manganese complexes of curcumin and its derivatives: evaluation for the radical scavenging ability and neuroprotective activity. Free Radical Biol. Med. 35: 1632–44.

Vallianou, N.G., Evangelopoulos, A., Schizas, N. and Kazazis, C. 2015. Potential anticancer properties and mechanisms of action of curcumin. Anticancer Res. 35: 645–651.

Vyas, K. 2015. The cure is in the roots: turmeric. J. Nutr. Disorders Ther. 5: 163.

Wahlstrom, B. and Blennow, G. 1978. A study on the fate of curcumin in the rat. Acta Pharmacol. Toxicol. 43(2): 86–92.

Wang, J., Du, X.X., Jiang, H. and Xie, J.X. 2009. Curcumin attenuates 6-hydroxydopamine-induced cytotoxicity by anti-oxidation and nuclear factor-kappa B modulation in MES23.5 cells. Biochem. Pharmacol. 78: 178–83.

Wang, L., Chen, X. and Du, Z. 2017. Curcumin suppresses gastric tumor cell growth via ROS-mediated DNA polymerase γ depletion disrupting cellular bioenergetics. J. Exp. Clin. Canc. Res.: CR. 36:47. doi:10.1186/s13046-017-0513-5.

Wang, M.S., Boddapati, S., Emadi, S. and Sierks, M.R. 2010. Curcumin reduces alpha-synuclein induced cytotoxicity in Parkinson's disease cell model. BMC Neurosci. 11: 57–67.

Wang, S.L., Li, Y., Wen, Y., Chen, Y.F., Na, L.X., Li, S.T. and Sun, C.H. 2009. Curcumin, a potential inhibitor of up-regulation of TNF-alpha and IL-6 induced by palmitate in 3T3- L1 adipocytes through NF-kappaB and JNK pathway. Biomed. Environ. Sci. 22: 32–9.

Wang, X., Kim, J.R., Lee, S.B., Kim, Y.J., Jung, M.Y., Kwon, H.K and Ahn, Y.J. 2014. Effects of curcuminoids identified in rhizomes of *Curcuma longa* on BACE-1 inhibitory and behavioral activity and lifespan of Alzheimer's disease Drosophila models. BMC Complem. Altern. M. 14: 88.

Wang, Z.S., Chen, L.Z., Zhou, H.P., Liu, X.H. and Chen F.H. 2017. Diarylpentadienone derivatives (curcumin analogues): Synthesis and anti-inflammatory activity. Bioorg. Med. Chem. Lett. 27(8): 1803–1807.

Williums, H.C., Dellavalle, R.P. and Garner, S. 2012. Acne vulgaris. Lancet 379: 361–372.

Xu, Y., Ku, B., Cui, L., Li, X., Barish, P.A., Foster, T.C. and Ogle, W.O. 2007. Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotrophic factor expression in chronically stressed rats. Brain Res. 1162: 9–18.

Yach, D., Hawkes, C., Gould, C.L. and Hofman K.J. 2004. The global burden of chronic diseases overcoming impediments to prevention and control. J. Amer. Med. Assoc. 291(21): 2616–2622.

Yang, F., Lim, G.P., Begum, A.N., Ubeda, O.J., Simmons, M.R., Ambegaokar, S.S., Chen, P.P., Kayed, R., Glabe, C.G., Frautschy, SA. and Cole, G.M. 2005. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. J. Biol. Chem. 280: 5892–901.

Yang, J.Y., Zhong, X., Yum, H.W., Lee, H.J., Kundu, J.K., Na, H.K. and Surh, Y.J. 2013. Curcumin inhibits STAT3 signaling in the colon of dextran sulfate sodium-treated mice. J. Cancer Prev. 18(2): 186–91.

Yang, Z., Zhao, T., Zou, Y., Zhang, J.H. and Feng, H. 2014. Curcumin inhibits microglia inflammation and confers neuroprotection in intracerebral hemorrhage. Immunol. Lett. 160(1): 89–95. doi:10.1016/j.imlet.2014.03.005.

Yao, E.C. and Xue, L. 2014. Therapeutic Effects of Curcumin on Alzheimer's Disease. Adv. Alzheimer Dis. 3(1): 145–159.

Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E.R. and Mizuno, Y. 1996. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc. Natl. Acad. Sci. USA 93: 2696–701.

Zari, S. and Zari, T. 2015. A review of four common medicinal plants used to treat eczema. J. Med. Plant. Res. 9(24): 702–711.

Zbarsky, V., Datla, K.P., Parkar, S., Rai, D.K., Aruoma, O.I. and Dexter, D.T. 2005. Neuroprotective properties of the natural phenolic antioxidants curcumin and naringenin but not quercetin and fisetin in a 6-OHDA model of Parkinson's disease. Free Radical Res. 39: 1119–25.

Zhang, Y., Liang, D., Dong, L., Ge X., Xu, F., Chen W., Dai, Y., Li, H., Zou, P., Yang, S. and Liang, G. 2015. Anti-inflammatory effects of novel curcumin analogs in experimental acute lung injury. Respir. Res. 16: 43–55.

Zhu, Y.G., Chen, X.C., Chen, Z.Z., Zeng, Y.Q., Shi, G.B., Su, Y.H. and Peng, X. 2004. Curcumin protects mitochondria from oxidative damage and attenuates apoptosis in cortical neurons. Acta Pharmacol. Sin. 25: 1606–12.

9

Medicinal Plants of the Khyang Tribe of Bandarban District, Bangladesh

Md. Shahadat Hossan,[1] *Christophe Wiart,*[2] *Emratul Jannat,*[3] *Abu Hanif,*[4] *Prozzal Roy,*[5] *Taufiq Rahman,*[6] *Rownak Jahan,*[7] *Tracey D. Bradshaw*[1] and *Mohammed Rahmatullah*[8,*]

Introduction

The Khyangs are a small ethnic group of people inhabiting the Chittagong Hill Tracts region in the southeast part of Bangladesh. At present, they can be mainly found in Rangamati and Bandarban districts of Chittagong Hill Tracts. Their total population is estimated to be around 2500. The Khyangs possibly came to their present habitat from neighboring Burma (presently Myanmar). They are considered by anthropologists as belonging to the Kuki-Chin race of the Tibeto-Burman group. Khyang societies are headed by a leader for each society, the leader being known as a 'Karbari'.

According to the Khyang elders, once upon a time they were nature worshippers but later converted to Buddhism and presently Christianity. Khyang society is patriarchal. Their method of cultivation is known as 'jhum', where a forest area is burnt completely followed by cultivation on that area for 2–3 years and then moving on to a new area and letting the previous area to revert back slowly to a forested state.

Humans have recognized the need for medicines to treat and cure diseases since their advent. It has been reported that humans were aware of medicinal properties of plants, possibly as early as 3000 BCE (Sofowara 1982). Historical surveys have indicated that the eastern region of the Mediterranean may always have been a rich source of medicinal plants and that indigenous Arab medicine was a major contributor to the development of modern medicine in Europe (Saad et al. 2005). The Edwin Smith Papyrus and the Ebers Papyrus indicate that the Egyptians were using herbal medicine during the 17th and 16th centuries BCE (El-Soud 2010). Ayurveda (India), Greek and Chinese traditional medicine also developed from around 2000 to 4000 years ago (Subbarayappa 2001).

[1] School of Pharmacy, University of Nottingham, UK.
[2] School of Pharmacy, University of Nottingham Malaysia Campus, Malaysia.
[3] BDS, Pioneer Dental College and Hospital, Dhaka, Bangladesh.
[4] Onehousing Group, London, UK.
[5] Zuellig Pharma Bangladesh Limited, Bangladesh.
[6] Department of Pharmacology, University of Cambridge, Tennis Court Road, CB2 1PD, UK.
[7] Department of Biotechnology & Genetic Engineering, University of Development Alternative, Dhaka, Bangladesh.
[8] Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh.
* Corresponding author: rahamatm@hotmail.com

In modern times, it has been recognized that the ancient medicinal knowledge of human beings can play a vital role in the discovery of efficacious drugs (Balick and Cox 1996). Since tribes and various indigenous people have used medicines for a long time period, they are usually the best knowledge holders of ancient forms of cure. It has also been recognized that phytotherapeutic practices, that is treatment of diseases with plants is the most common form of practice among indigenous people, possibly partly because of the abundance of plants, and partly because any given plant may possess hundreds of phytochemicals with potential disease curing ability. Moreover, plants are affordable and can yield affordable medicines in the form of simple decoctions or pastes, which can be simply used either orally or topically.

As such, it is important to document the medicinal practices of tribes before they get totally lost due to the onslaught of 'civilization' and 'globalization'. Bangladesh is possibly home to over a hundred small or large tribes. However, ethnomedicinal data on these tribes are practically negligible. We have previously documented the traditional medicinal practices of folk medicinal practitioners and a few tribes of Bangladesh (Akter et al. 2014, Azad et al. 2014, Islam et al. 2015a,b, Rahman and Rahmatullah 2015, Zaman et al. 2015, Seraj et al. 2017). The present chapter gives an account of the traditional medicinal practices of Khyang Tribal Medicinal Practitioners (TMPs) residing in Khyaplong-para and Shuanlo-para in Rowangchaari of Bandarban district, Bangladesh. To our knowledge, there has been scant or no information previously on medicinal plants of the Khyang.

Loss of Khyang traditional medicinal practices including their phyto-therapeutic knowledge may prove to be disastrous in the long run because along with ongoing rapid deforestation, this may lead to loss of both medicinal plants and their uses. Discovery of important and vital drugs may suffer from these dual losses. The Khyangs have inhabited their present area for a long time and so are most knowledgeable about the local plant species. The aim of the present study was to document the Khyang tribal medicinal practices involving mostly plants before such knowledge and plant species become irretrievably lost.

Ethnomedicinal survey

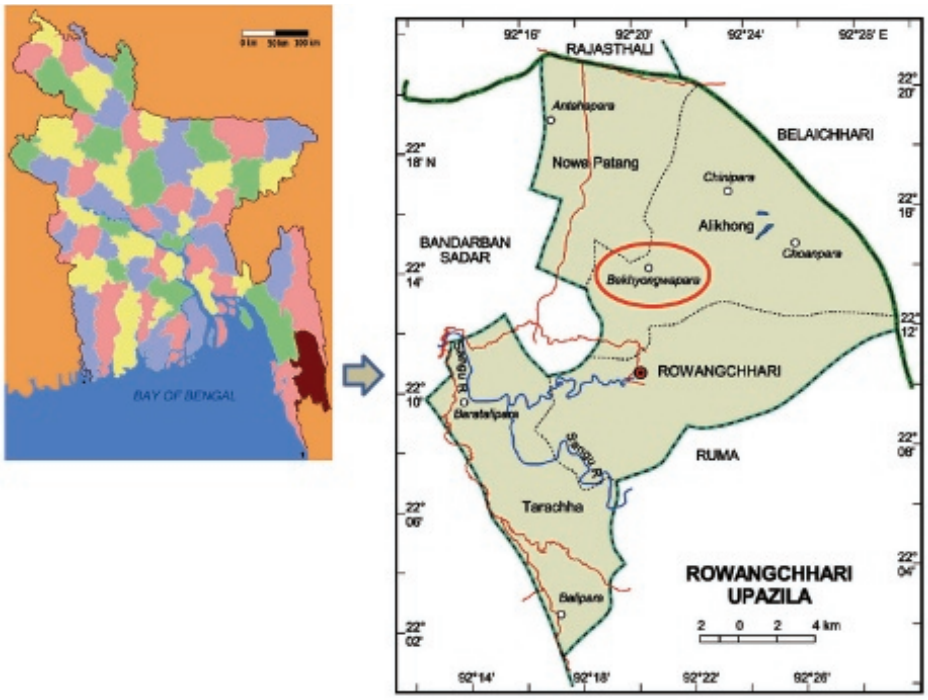
Ethnomedicinal survey is essentially the tool to collect data on tribal medicinal plants and their uses. The present data was obtained from the Khyang tribal medicinal practitioners (TMPs) practicing among the Khyang communities of Khyaplong-para and Shuanlo-para in Rowangchaari of Bandarban district, Bangladesh (Fig. 9.1) through such a survey. Altogether, a total of 15 Khyang TMPs were interviewed, thirteen of them being males and two females. The names and other details of the TMPs are given in Table 9.1. The ages of the TMPs ranged from 37–70 years, suggesting that even today these Khyang communities have not given up on their traditional medicinal treatments.

Ethnomedicinal information is generally collected through interviews of tribal TMPs, the present study being no exception. Interviews were conducted with the help of a semi-structured questionnaire and the guided field-walk method of Martin (1995) and Maundu (1995). In this method, the TMPs took the interviewers on guided field-walks both individually and collectively through areas from where they collected their medicinal plants or plant parts (Fig. 9.2), pointed out the plants, and described their uses.

Plant species used by the Khyangs

The Khyang TMPs were observed to use 84 plant species in their treatment. The names of the plant species along with other taxonomic information are shown in Table 9.2. Khyang treatment appeared to be basically monoherbal, with one whole plant or plant part being used to treat a single or multiple disease(s). The formulations and treatment modes were also fairly simple with plant paste, decoction or juice being taken either orally or applied topically. Gastrointestinal disorders in the form of dysentery, blood dysentery, and diarrhea were the most common ailments treated. However, the TMPs had phytomedicines for some serious ailments (by serious is meant that existing allopathic medicines do not bring total cure) like heart disorders, arthritis, rheumatism, hypertension, tumor, cancer, and diabetes.

Surprisingly, although the forested regions of the Chittagong Hill Tracts are known for prevalence of malaria (Starzengruber et al. 2014) including cerebral malaria, the Khyang TMPs did not mention malaria among the diseases treated. The TMPs, on the other hand, treated fever, which may be a symptomatic



Maps adopted from:
http://lib.pmo.gov.bd/maps/images/bandarban/Rowangchhari.gif
https://upload.wikimedia.org/wikipedia/commons/8/8a/Bangladesh_Bandarban_District.png

Fig. 9.1 Map showing Bangladesh (left) and study area (right).

Table 9.1 Information on the interviewed Khyang tribal healers.

Name of traditional healer	Age	Gender	Area
Aung-marrak	60	M	Khyaplong-para
Chaillong Khyang*	58	M	Khyaplong-para
Paichong	70	M	Khyaplong-para
Paisra	37	F	Khyaplong-para
Prothoai Khyang (Karbari)*	63	M	Khyaplong-para
Rathung	51	M	Khyaplong-para
Shalashu	49	M	Shuanlo-para
Shala-u khyang	39	M	Shuanlo-para
Shoikh-la	44	M	Shuanlo-para
Sialian Khyang*	52	F	Khyaplong-para
Timraw	53	M	Khyaplong-para
Tinglaw	66	M	Shuanlo-para
Tonlapru	55	M	Shuanlo-para
Unshalank	40	M	Khyaplong-para
Vanlalshia	60	M	Khyaplong-para

*Principal healers

Fig. 9.2 Collection of medicinal plants in guided field-walks with Khyang TMPs.

treatment of malaria, for malaria causes fever. It is also interesting in this aspect that fever, including fever with convulsions (this type of fever may be malarial fever) was treated with a number of different plant species, which indicates the importance given by the TMPs to treat fever.

Selected phytochemical constituents of Khyang medicinal plants and their pharmacological activities

Table 9.3 gives an account of the important phytoconstituent(s) present in the Khyang medicinal plants and shows that although a large number of medicinal plants used by the Khyang TMPs are yet to be investigated for their phytochemical constituents, the plants that have been investigated possess bioactive compounds relevant to their medicinal uses plus possibly other therapeutic uses. For instance, one of the plants used to treat tumor was *Amorphophallus paeoniifolius* (Dennst.) Nicolson. The plant contains quercetin; the compound can reverse pre-neoplastic lesions caused by chemical carcinogenesis (Carrasco-Torres et al. 2017). Quercetin also reportedly induces apoptosis and necroptosis in MCF-7 breast cancer cells (Khorsandi et al. 2017). To give just one more example, *Ocimum americanum* was also used by the Khyang TMPs to treat tumors. The anti-proliferative activity of the essential oils obtained from this plant collected from Burkina Faso has been described (Bayala et al. 2014). Moreover, the plant contains rosmarinic acid, which may be a good anti-cancer agent (Dall'Acqua et al. 2017). The plant furthermore contains ursolic acid, which reportedly can inhibit hepatocellular carcinoma (Liu et al. 2017, Zhao et al. 2017). The structures of some important phytoconstituents present in the Khyang medicinal plants are shown in Fig. 9.3. The reported pharmacological activities of some of the important phytoconstituents are discussed (below). This following discussion will be essentially based on reviews of individual phytoconstituent instead of describing every individual paper dealing with any given pharmacological activity of the phytoconstituent.

***Abelmoschus moschatus* Medik.** Myricetin, present in the plant, reportedly possesses anti-oxidant, anti-carcinogen, anti-viral, anti-diabetic, anti-thrombotic, anti-microbial activities, and useful in the treatment of gout and diarrhea (Ong and Khoo, 1997).

***Acacia catechu* (L.f.) Willd.** Various biologically active phytocomponents present in the plant are Catechin, Epicatechin, Epicatechin gallate, Procatechinic acid, Quercetin, Rutin, and Kaempferol. Catechin, epicatechin, and epicatechin gallate possess anti-cancer, hypocholesterolemic and hypotensive activities (Sonoda et al. 2015). Quercetin is antioxidant and can be beneficial in cancer, allergy, asthma, diabetes, cardiovascular diseases, oral mucosa (mouse apthous ulcers), gastric ulceration, hypertension, immunity and

Table 9.2 Medicinal plants and formulations of the Khyang tribe.

Serial No.	Botanical name	Family name	Local name	Ailment/medical use	Part utilized	Mode of preparation (if known)
1.	*Abelmoschus moschatus* Medik.	Malvaceae	Vomla/Mishun	Aphrodisiac, Stress, Depression, Sore throat, Throat pain, Tonsillitis, Scabies	Leaf, Root, Seed	Juice obtained from leaf and root is taken orally. Seed and root paste is applied topically for pain or scabies.
2.	*Acacia catechu* (L.f.) Willd.	Fabaceae	Khangmui	Acne, Leprosy, Gum pain, Chronic blood dysentery	Fruit, Leaf	Infusion obtained from leaf and fruit is taken orally. Fruit juice is applied externally.
3.	*Acacia farnesiana* (Linn.) Willd.	Fabaceae	Hoija	Leprosy, Gum pain	Bark, Leaf	Dried leaf and bark powder or concentrated infusion of leaf and bark is applied for 7-10 days topically.
4.	*Achyranthes aspera* L.	Amaranthaceae	Saikka	Jaundice, Flatulence, Expectorant, Constipation, Excessive bleeding during menstruation, Heart disease, Coagulant	Root, Leaf	Root powder is taken with honey, daily 1 spoon twice for 5–7 days. Leaf juice/squeezed leaf applied as coagulant.
5.	*Acorus calamus* L.	Acoraceae	Thith	Toothache	Root	Fresh root juice is applied.
6.	*Ageratum conyzoides* (L.) L.	Asteraceae	Urr	Antiseptic, Blood coagulant	Leaf	Squeezed leaf applied to cut area and held for a while.
7.	*Allophylus cobbe* (L.) Räusch.	Sapindaceae	Natokthum	Ulcer, Gastrointestinal tract (GIT) problem	Root	Decoction of root taken orally.
8.	*Alpinia conchigera* Griff.	Zingiberaceae	Soilopropat	Stomach ache, Boils, Wound, Swelling, Inflammation	Leaf, Root	Leaf juice taken for stomach ache. Root paste applied topically or rubbed.
9.	*Alstonia scholaris* (L.) R.Br.	Apocynaceae	Shijgi	Acne, Blood dysentery, Anthelmintic, Leprosy	Bark, Flower, Exudates	Acne (exudates rubbed), Blood dysentery (bark juice taken orally), Anthelmintic (bark juice taken orally), Leprosy (exudates rubbed).
10.	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Araceae		Boil, Piles, Tumor, Inflammation, Bronchitis, Premature ejaculations, Aphrodisiac	Root	Root paste is applied and infusion of root is taken orally.
11.	*Antidesma acuminatum* Wall.	Phyllanthaceae	Chiamchi	Anaemia, Constipation	Fruit	The fruit salad can be eaten with food/meal as side daily once for one week to treat anaemia and constipation.

12.	*Argyreia capitiformis* (Poir.) Ooststr.	Convolvulaceae	Lungmulthur	Insect bite, Anti-poisonous, Itch, Scabies, Bone fracture, Fever	Root, Leaf	Leaf and root paste is rubbed on the affected area to treat insect bite, as anti-poison, and for itch, scabies, and bone fracture.
13.	*Aristolochia bracteolata* Linn.	Aristolochiaceae	Aan-h-lum	Convulsion in children, Fever	Leaf	Infusion of leaf is boiled and cooled; the infusion is then used for shower.
14.	*Bauhinia acuminata* Linn.	Fabaceae	Laldir	Epilepsy, Cough, Jaundice, Cold, Vermifuge	Root	The decoction of the root is used to gargle to get relief from cough and cold. One tea spoon decoction can be taken orally with other drinks or water for 5–7 days once daily to treat jaundice, epilepsy, and as vermifuge.
15.	*Begonia barbata* Wall.	Begoniaceae	Kukthur (red)	Diarrhea of children	Leaf, Stem	Obtained juice from leaf and stem is taken 3 times with breast milk for one day.
16.	*Begonia silhetensis* (A. DC.) C.B. Clarke	Begoniaceae	Kukthur (white)	Diarrhea of children	Leaf, Stem	Obtained juice from leaf and stem is taken 3 times with breast milk for one day.
17.	*Belamcanda chinensis* (L.) DC.	Iridaceae	Lily	Breathing problem, Skin disorder	Root, Leaf	Root and leaf extract (prepared by boiling with water and concentrating) is applied topically for skin disorder and infusion is taken orally for breathing problem.
18.	*Breynia retusa* (Dennst.) Alston	Phyllanthaceae	Fulamulthir	Cough, Flu, Toothache, Dysentery, Conjunctivitis	Fruit, Root, Leaf	Juice obtained from fruit & leaves taken orally for dysentery & cough, root juice topically applied for toothache and conjunctivitis.
19.	*Calotropis procera* (Ait.) Ait.f.	Asclepiadaceae	Muru-o	Joint pain, Arthritis, Muscle pain	Leaf	Leaves are warmed and applied to painful areas.
20.	*Cassia alata* L.	Fabaceae	Rulrijaw/ Builakung	Ringworm, Eczema	Leaf	Leaf juice is applied topically.
21.	*Casuarina equisetifolia* L.	Casuarinaceae	Farthing	Beriberi, Diarrhea, Swelling, Astringent	Bark	Bark paste is applied topically for swelling. Bark juice is also taken half spoon daily for 2 days in case of diarrhoea.
22.	*Centella asiatica* (L.) Urb.	Apiaceae	Mawtung	Stomach ache, Stomach fullness, Insect bite or poisoning due to insect bite	Whole plant	Whole plant is taken orally as chutney/salad.

Table 9.2 contd....

...Table 9.2 contd.

Serial No.	Botanical name	Family name	Local name	Ailment/medical use	Part utilized	Mode of preparation (if known)
23.	*Citrus aurantifolia* (Christm.) Swingle	Rutaceae	Raite	Anthelmintic, Headache, Skin disorder	Fruit, Leaf	Juice of fruit and leaf taken orally.
24.	*Clausena suffruticosa* Wight & Arn.	Rutaceae	Changeerathing	Arthritis, Joint pain	Root, Bark	Bark and root paste is rubbed topically.
25.	*Clerodendrum indicum* (L.) Kuntze.	Verbenaceae	Aanfui	Tranquilizer, Mental illness	Leaf, Root	Paste of root and leaf is applied on top of head. Clean leaves and roots are boiled with water and the water used to take a bath to cure mental illness.
26.	*Clerodendrum viscosum* Vent.	Verbenaceae	Koidim	Nausea, Vomiting, Post-partum diarrhea and burning in the hand and feet	Leaf	Half tea spoon of leaf juice is taken orally twice daily for 3 days.
27.	*Cocos nucifera* L.	Arecaceae	Unchitai	Anthelmintic, Malaria, Aphrodisiac, Vermifuge	Fruit, Leaf	Fresh coconut meat is taken orally. Coconut water is taken orally to treat all of these diseases.
28.	*Curculigo recurvata* Dryand.	Hypoxidaceae	Nathial	Aphrodisiac	Rhizome	Rhizome juice is taken orally.
29.	*Cuscuta reflexa* Roxb.	Cuscutaceae	Chokro	Jaundice, Liver disease, Uterus and Liver pain	Stem	Obtained juice from stem is taken orally. Stem is cooked and taken orally as curry.
30.	*Cyperus difformis* L.	Cyperaceae	Thit	Mental problem, Tranquilizer	Root	Infusion of root used for shower. Root juice is taken orally.
31.	*Delonix regia* (Bojer) Raf.	Fabaceae	Sandri	Antibacterial, Rheumatism, Fungal infection	Root, Bark	Paste of root and bark is topically applied.
32.	*Dillenia indica* L.	Dilleniaceae	Aitlung	Edible, Blood dysentery, Flu and fever	Fruit	Fruits are eaten as pickle. Ripe fruit is taken orally.
33.	*Dioscorea glabra* Roxb.	Dioscoreaceae	Breng	Astringent, Jaundice, Topical pain	Rhizome, Leaf	Rhizomes are cooked and eaten as curry, leaf juice is taken orally in case of jaundice, crushed leaf is applied topically to pain area to get relief from pain.
34.	*Dioscorea pentaphylla* L.	Dioscoreaceae	Manshimoklok	Stomach ache, Leprosy, Fever	Leaf	Leaf juice is taken orally for stomach ache and fever and applied topically for leprosy.
35.	*Dioscorea* sp.	Dioscoreaceae	Toalrang	Snake bite, Insect bite, Anti-poisonous	Fruit	Juice obtained from fruit is applied or rubbed on affected area.
36.	*Elephantopus scaber* L.	Asteraceae	Mrangkhua	Heal pain, Heal inflammation	Leaf	Heated leaf is applied to the heal after warming.

37.	*Erythrina variegate* L.	Fabaceae	Faiseo	Worm infection	Root	1 teaspoon root juice is taken daily once for 3 days.
38.	*Eupatorium odoratum* L.	Asteraceae	Pache-ye	Astringent, Coagulant	Leaf	Squeezed leaf applied to cut area and held for a while.
39.	*Euphorbia hirta* L.	Euphorbiaceae	Dudhothe	Scabies, Wound, Itch	Leaf	Leaves are boiled in water and the water used to take a bath.
40.	*Globba marantina* L.	Zingiberaceae	Aienghang	Malaria, Typhoid, Chronic fever, Kala azar, Fever with extreme convulsions	Rhizome	Juice obtained from rhizome is orally taken (1 tea spoon daily twice for 2 days).
41.	*Gymnopetalum cochinchinense* (Lour.) Kurz.	Cucurbitaceae	Mansonkhirat/ Aankhate/ Tho-umpong	Acidity, GIT problem, Leucorrhoea	Fruit	Infusion obtained from dried crushed fruit is taken orally.
42.	*Hedyotis scandens* Roxb.	Rubiaceae	Taruthing	GIT problem, Boil, Stomach ache	Leaf	Paste obtained from leaf is applied topically for boils. Juice/Infusion obtained from leaves is taken orally for GIT problem or stomach ache.
43.	*Hoya parasitica* Wall.	Asclepiadaceae	Nempar	Fever, Body ache	Leaf	Leaf juice or paste of leaf is used externally.
44.	*Hyptis suaveolens* (L.) Poit.	Lamiaceae	Baish-rung	Stomach fullness, GIT	Seed	Overnight soaked seed in water is taken orally.
45.	*Ixora parviflora* Vahl	Rubiacea	Toalchu	Menstrual pain	Root	Crushed root powder + Vomla (*Abelmoschus moschatus*) plant's crushed root powder is mixed with 1 tea spoon water and taken orally.
46.	*Kalanchoe pinnata* (Lam.) Pers.	Crassulaceae	Nasirkhaw	Cough, Cold, Flu	Leaf	Leaf is heated over fire for 1–2 minutes and then squeezed to obtain juice, which is taken orally. Dose: 2 tea spoons twice daily for 3 days.
47.	*Lygodium flexuosum* (L.) Sw.	Lygodiaceae	Paingjemjit	Increasing of breast milk, Loss of appetite, Diuretic, Ear infection	Leaf	Leaf juice is taken with coconut milk to increase breast milk for breast feeding mother. Leaf juice is taken orally for loss of appetite and as diuretic. Leaf juice is applied in the ear for otitis (ear infection).
48.	*Mikania cordata* (Burm.f.) B.L. Rob.	Asteraceae	Bache-a	Coagulant, Astringent	Leaf	Leaf is squeezed and applied to cut area.

Table 9.2 contd....

...Table 9.2 contd.

Serial No.	Botanical name	Family name	Local name	Ailment/medical use	Part utilized	Mode of preparation (if known)
49.	*Mimosa pudica* L.	Fabaceae	Beljak	Constipation, Acidity, Gingivitis	Leaf, Root	Juice obtained from leaf and root is taken orally.
50.	*Moghania macrophylla* (Willd.) Kuntze	Fabaceae	Thialai-padarkep	Arthritis, Joint pain	Leaf	Leaf warmed by heat is applied on pain-affected area.
51.	*Morinda angustifolia* Roxb.	Rubiaceae	Jongmit	Bone fracture, Arthritis, Inflammation	Leaf	Leaf paste is applied topically.
52.	*Mucuna gigantean* (Willd.) DC.	Fabaceae	Bybra	Astringent, Blood coagulant	Leaf	Leaf juice is applied to cut area.
53.	*Mussaenda corymbosa* Roxb.	Rubiaceae	Kuthbul	Tumor, Astringent	Root, Leaf	Extract of root and leaf is applied topically.
54.	*Nymphaea nouchali* Burm.f.	Nymphaeaceae	Ba-aa	Anaemia, Bile disease, Menstruation problem	Leaf, Rhizome	Boiled leaf and rhizome is taken orally with food.
55.	*Ocimum americanum* L.	Lamiaceae	Vipena pang	Tumor, Infection, Ulcer, Cancer	Leaf, Aerial part	Aerial part or leaf juice is applied externally for infection and taken orally for ulcer, cancer and tumour.
56.	*Opuntia dillenii* Ker-Gawl.	Cactaceae	Renkung	Asthma, Anti-poisonous, Insect bite, Rheumatism	Leaf, Exudates	Leaf juice is taken orally for asthma. Exudates applied topically for insect bite and rheumatism.
57.	*Phyllanthus emblica* L.	Phyllanthaceae	Shuanlu	At the time of urination pain in lower abdomen, Burning sensation in urinary tract, Leucorrhoea	Fruit	Fruit juice mixed with rice washed water is taken orally until cure.
58.	*Premna scandens* Roxb.	Verbenaceae	Aan-norai	Neurological disorder, Stress, Tranquilizer	Leaf	Young leaves are eaten as chutney/salad.
59.	*Psychotria calocarpa* Kurz.	Rubiaceae	Shipujo-o	Abscess, Scabies	Root	Root paste is applied or rubbed topically.
60.	*Sansevieria roxburghiana* Schult. and Schult.f.	Asparagaceae	Faackrethal	Otitis, Wound, Scabies	Leaf	Crushed leaf is rubbed on affected area.
61.	*Sarcochlamys pulcherrima* (Roxb.) Gaud.	Urticaceae	Naplung	Fever, Gout, Goitre	Leaf, Root	Infusion obtained from leaf is taken orally.
62.	*Scoparia dulcis* L.	Scrophulariaceae	Turojot-turpang	Fever (children), Stomach ache, Dysentery	Root	Root juice at low concentration is taken with breast milk for fever in children. For stomach ache and dysentery in adults, fresh juice is taken twice daily for 2–3 days.
63.	*Selaginella decipiens* Warb.	Selaginellaceae	Shikithing	Gout, Vertigo	Whole plant	Whole plant decoction is used to rub on the painful area.

64.	*Smilax macrophylla* Willd.	Smilacaceae	Wisisong	Toothache	Root	Root juice is applied on affected tooth.
65.	*Solanum lasiocarpum* Dunal	Solanaceae	Shialmandak	Mental disorder, Scabies, Piles, Allergy in eye, Leprosy, Fever, Cough, Nausea	Leaf	Leaf juice or decoction is mixed with bath water while bathing to treat scabies, piles, leprosy, allergy, fever and the rest of the ailments.
66.	*Solanum torvum* Sw.	Solanaceae	Mirem	Blood dysentery, Low Blood pressure, Arthritis	Leaf, Root	Decoction of root is taken half tea spoon daily for 3–5 days with meal for dysentery and blood pressure. Leaf juice or leaf paste is used topically for arthritic pain.
67.	*Spilanthes calva* DC.	Asteraceae	Aankhasaai	Toothache	Flower	Flower paste is applied to affected area.
68.	*Spondias pinnata* Kurz	Anacardiaceae	Thoailo-o	Wound, Otitis	Fruit, Bark	Juice obtained from fruit and bark is applied into the ear for otitis and topically applied to wounds.
69.	*Spondias cytherea* Sonn.	Anacardiaceae	Thoai-h-lok	Excessive bleeding during menstruation	Root	Juice obtained from root is taken orally.
70.	*Synedrella nodiflora* (L.) Gaertn.	Asteraceae	Man-ankhasha	Anthelmintic	Whole plant	Infusion of whole plant is taken 1 spoon orally 2–3 times a day for 3 days.
71.	*Syzygium cumini* (L.) Skeels	Myrtaceae	Bulbu	Diabetes, Dysentery, Antiemetic	Seed	Dried crushed seed powder taken orally 2 spoons daily for four weeks.
72.	*Tabernaemontana divaricata* (L.) R. Br. Roxb.	Apocynaceae	Sisakrai	Leprosy	Fruit	Powder of dry fruit applied topically.
73.	*Tamarindus indica* L.	Fabaceae	Theng-ther-le	Conjunctivitis, Loss of appetite, Inflammation	Fruit	Ripe fruit pulp is eaten for loss of appetite. Diluted (1:5) leaf juice is used for eye disease. Leaf paste is applied topically for inflammation.
74.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Thakbraw	Expectorant, Conjunctivitis, Asthma, Aphrodisiac	Fruit	Infusion of fruit is taken orally.
75.	*Terminalia chebula* Retz.	Combretaceae	Thabra	Cough, Diarrhea	Bark, Seed	Seed pulp is taken orally for diarrhoea. Bark juice is taken orally for cough.
76.	*Thunbergia grandiflora* Roxb.	Acanthaceae	Jongrikhaw	Eye problem, Cut, Astringent, Wound	Leaf, Stem	Leaf and stem juice is taken orally as astringent or applied topically.
77.	*Thysanolaena maxima* (Roxb.) Kuntze	Poaceae	Jaruthing	Boil, Abscess, Cancer (externally visible tumor)	Leaf	Leaf paste is applied topically.

Table 9.2 contd....

...Table 9.2 contd.

Serial No.	Botanical name	Family name	Local name	Ailment/medical use	Part utilized	Mode of preparation (if known)
78.	*Trichosanthes tricuspidata* Lour.	Cucurbitaceae	Hedopang/ Mokal	Tumor, stomach ulcer, cancer	Fruit, Seed	Sun dried fruits and seed are soaked in tribal wine for 3 days; this wine is then taken orally 1 table spoon daily at night for 2–3 months.
79.	*Urena lobata* L.	Malvaceae	Pethai/Koptorik	Fever, Cold sore, Aphthae	Leaf, Root	Leaf juice is applied externally/topically for cold sore and aphthae. Infusion of leaf and root is used for shower to cure fever.
80.	*Vitex negundo* L.	Verbenaceae	Namturpung	Expectorant, Flatulence, Stress, Headache, Leprosy, Anthelmintic, Antipyretic	Fruit, Leaf	Fume from burning dried leaf is inhaled for headache and Stress. Dried fruit powder is taken orally for worms and fever. Dried fruit powder is applied topically for leprosy treatment.
81.	*Vitis pedata* L.	Vitaceae	Jolpol	Jaundice, Neurological problem, Tranquilizer	Leaf	Leaf is cooked and taken as vegetable or curry (cooked with hilly crab).
82.	*Vitis pentagona* (Roxb.) Lawson	Vitaceae	Dingdung	Vertigo, Tranquilizer	Leaf	Leaf juice is warmed and after cooling topically applied to scalp.
83.	*Zingiber officinale* Roscoe	Zingiberaceae	Tumreng	Blood pressure, Dyspnoea, Acidity, Stomach upset	Rhizome	Juice obtained from rhizome is taken orally.
84.	*Ziziphus mauritiana* Lam.	Rhamnaceae	Merai	Dysentery with blood and mucus in stool, Edible	Bark, Fruit	Bark juice is taken orally. Fruits are eaten.

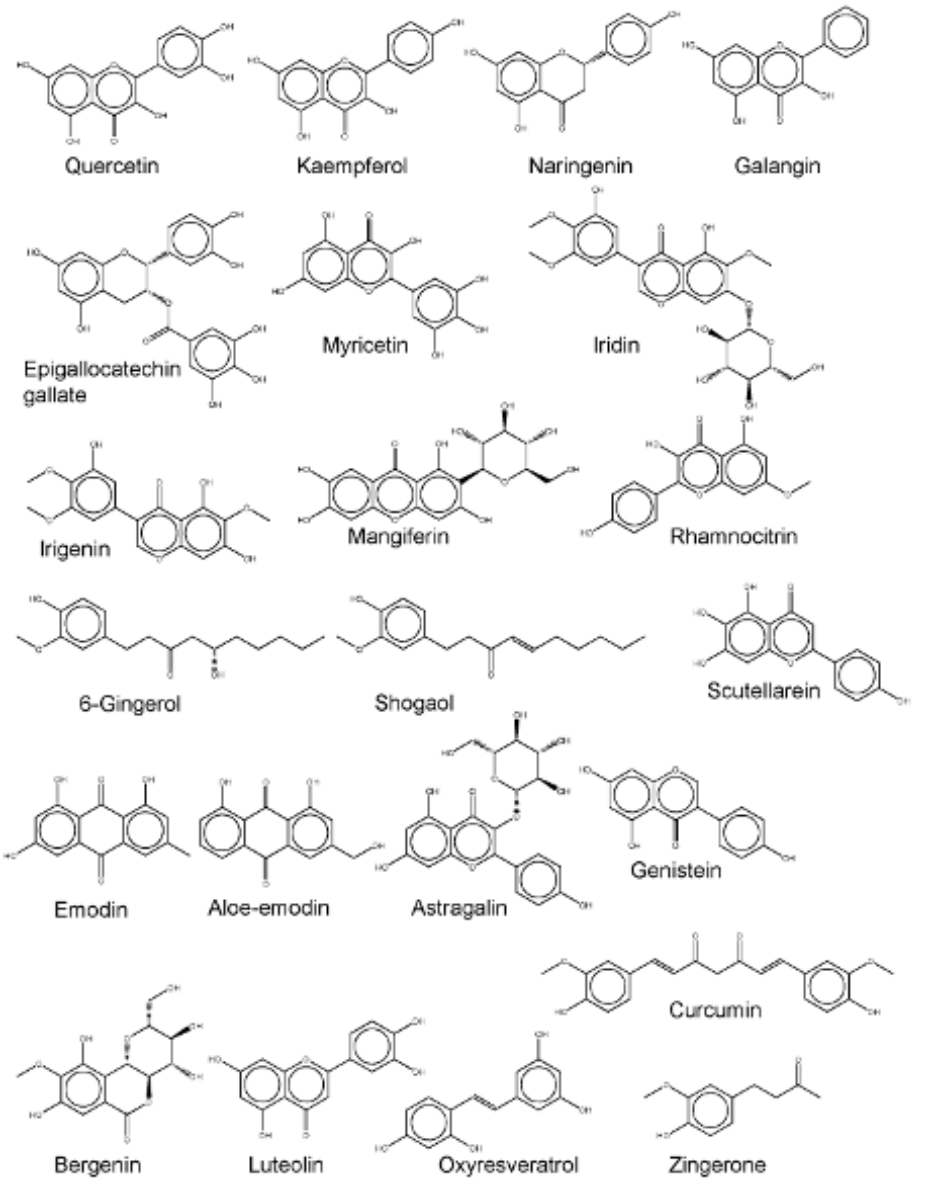


Fig. 9.3 Bioactive phenolic present in various plants used by the Khyang tribe.

infections, inflammation, injury and pain, prostatitis/interstitial cystitis, arthritis, metabolic syndrome, and obesity (Kelly 2011). Rutin reportedly has pharmacological benefits against cancer, diabetes, hypertension, and hypercholesterolemia (Al-Dhabi et al. 2015). Kaempferol and some of its glycosides reportedly possess antioxidant, anti-inflammatory, anti-cancer, anti-microbial, cardioprotective, neuroprotective, anti-diabetic, anti-osteoporotic, anxiolytic, analgesic, and anti-allergic activities (Calderón-Montaño et al. 2011).

***Acacia farnesiana* (Linn.) Willd.** Phytochemical constituents include Gallic acid, Ellagic acid, Kaempferol, and Naringenin. Gallic acid has antioxidant and anti-cancer effects (Badhani et al. 2015). Ellagic acid has antioxidant and anti-microbial properties (Vattem and Shetty 2005). Naringenin has been found to have metal chelating property along with antioxidant, anti-microbial, anti-viral, anti-allergic, anti-estrogenic, anti-diabetic, anti-inflammatory, anti-obesity, anti-cancer, adipolytic and hepatoprotective activities. Moreover, the compound may be useful in hypoxia and ischemic heart disease (Vishnu Varthan et al. 2013).

***Achyranthes aspera* L.** Some reported phytoconstituents are Achyranthine, Oleanolic acid, and Ecdysterone. Oleanolic acid possesses hepatoprotective, anti-inflammatory, antioxidant, and anti-cancer properties (Pollier and Goossens 2012).

***Acorus calamus* L.** The various compounds mentioned in Table 9.3 for this plant possess anti-bacterial, anti-fungal, insecticidal, and nematicidal activities; additionally, the plant or plant extract has in laboratory experiments shown anti-inflammatory, anti-adipogenic, insulin sensitization, immunomodulatory, neuroprotective, antioxidant, broncho-dilatory, and anti-proliferative effects, suggesting that more bio-active components may be present (Mythili Avadhani et al. 2013). 1,8-Cineole has shown anti-neoplastic, acaricide, allelopathic, anthelmintic, anti-allergic, anti-bronchitic, anti-catarrh, anti-fatigue, anti-laryngitic, anti-pharyngitic, anti-inflammatory, anti-bacterial, and hypotensive effects (Ramya et al. 2012).

***Ageratum conyzoides* (L.) L.** Various pharmacological studies indicate that β-sitosterol can prove useful in heart disease, hypercholesterolemia, modulating the immune system, prevention of rheumatoid arthritis, cervical cancer, hair loss, and benign prostatic hyperplasia (Saeidnia et al. 2014).

***Alstonia scholaris* (L.) R.Br.** Betulinic acid reportedly has anti-retroviral, anti-malarial, anti-inflammatory, anti-cancer, and anti-tumor properties (Ramya et al. 2012).

***Aristolochia bracteolata* Linn.** Aristolochic acid can cause renal failure and urothelial carcinoma (Yang et al. 2014).

***Belamcanda chinensis* (L.) DC.** Pharmacological activity studies with mangiferin indicate that the compound may be antioxidant, analgesic, anti-diabetic, anti-proliferative, chemopreventive, radioprotective, cardiotonic, immunomodulatory, and diuretic (Jyotshna et al. 2016).

***Cassia alata* L.** Emodin has been reported to give anti-cancer, hepatoprotective, anti-inflammatory, antioxidant, and antimicrobial effects (Dong et al. 2016). Chrysophanol has been shown to demonstrate antioxidant and anti-inflammatory activities in microglia (Lin et al. 2015).

***Centella asiatica* (L.) Urb.** Together with madecassic acid and Asiatic acid, asiaticoside has wound healing effect; by itself, asiaticoside is anti-microbial, cytotoxic against hepatoma and melanoma, anxiolytic and neuroprotective; madecassoside reportedly can stimulate collagen synthesis (Alfarra and Omar 2013). Linalool reportedly has sedative, anxiolytic, analgesic, anti-convulsant, and anti-inflammatory effects (Aprotosoaie et al. 2014).

***Citrus aurantifolia* (Christm.) Swingle.** β-Caryophyllene is anti-microbial and cytotoxic (anti-cancer) (Neta et al. 2016), the compound is also hepatoprotective (Varga et al. 2017).

***Cocos nucifera* L.** Ferulic acid has been reported to have antioxidant, anti-microbial, anti-inflammatory, anti-thrombosis and anti-cancer properties (Ou and Kwok 2004). Amyrins are known to be anti-hyperglycemic and hypolipidemic (Santos et al. 2012).

***Cuscuta reflexa* Roxb.** Luteolin is an anti-inflammatory and neuroprotective agent (Nabavi et al. 2015). The compound is also reportedly antioxidant, anti-bacterial, anti-diabetic, and anti-proliferative and inhibits colorectal cancer (Pandurangan and Esa 2014).

***Elephantopus scaber* L.** Lupeol reportedly has beneficial effects against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity, and hepatotoxicity (Wal et al. 2011).

***Erythrina variegata* L.** Oxyresveratrol has anti-neoplastic, antioxidant, skin whitening, neuroprotective, hepatoprotective, and hypoglycemic activities (Xu et al. 2014).

***Eupatorium odoratum* L.** Chlorogenic acid has antioxidant, anti-inflammatory, cardioprotective, hepatoprotective, renoprotective, anti-diabetic, and anti-lipidemic activities (Maalik et al. 2016).

***Euphorbia hirta* L.** Reported activities of gallic acid include hepatoprotective, anti-cancer, anti-microbial, anti-inflammatory, anti-depressant, anti-Parkinson, anti-diabetic, anti-malarial, diuretic, wound healing, anthelmintic, and anxiolytic (Nayeem et al. 2016). Caffeic acid is carcinogen inhibitor, anti-oxidant, anti-bacterial, and cardioprotective (Magnani et al. 2014). p-Coumaric acid is antioxidant and anti-microbial

Table 9.3 Some notable bioactive phytochemicals in the Khyang medicinal plants.

Serial No.	Botanical name	Notable bioactive phytochemicals
1.	*Abelmoschus moschatus* Medik.	Myricetin
2.	*Acacia catechu* (L.f.) Willd.	Catechin; Epicatechin; Epicatechin gallate; Procatechinic acid; Quercetin; Rutin; Kaempferol
3.	*Acacia farnesiana* (Linn.) Willd.	Gallic acid; Ellagic acid; Kaempferol; Naringenin
4.	*Achyranthes aspera* L.	Achyranthine; Oleanolic acid; Ecdysterone
5.	*Acorus calamus* L.	1,8-cineole; α-Humulene; α-Pinene; α-Terpinene; (–)-α-Terpineol; Curcumene; Asarone; (–)-β-Elemene; Borneol; Elemicin; Eugenol; Limonene; Linalool; Menthol; Galangin
6.	*Ageratum conyzoides* (L.) L.	α-Pinene; α-Terpinene; (–)-β-Elemene; β-Sitosterol; Borneol; (–)-β-Caryophyllene epoxide; Eugenol; Farnesol; Friedelin; Limonene; Quercetin; Kaempferol
7.	*Allophylus cobbe* (L.) Räusch.)	Phenolics; Alkaloids; Cyanolipids
8.	*Alpinia conchigera* Griff.	1'S-1-acetoxychavicol acetate (ACA); various lignans such as Conchigeranals A-E, Galanganal, Galanganols A and B Sesquineolignans - Conchignans A, B, and C, together with Vanillin and Phloroglucinol Essential oils with Eucalyptol (25.85%); Chavicol (25.08%), β-Pinene (6.71%); Caryophyllene (3.38%) as the major components Cardamomin; 2',4'-Dihydroxy-6'-methoxychalcone
9.	*Alstonia scholaris* (L.) R.Br.	Betulin; Betulinic acid; Oleanolic acid; Ursolic acid; β-Sitosterol; β-Amyrin acetate; Corialstonine; Corialstonidine; Scholaricine; Vallesamine
10.	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Quercetin
11.	*Antidesma acuminatum* Wall.	Data not available to date
12.	*Argyreia capitiformis* (Poir.) Ooststr.	Data not available to date
13.	*Aristolochia bracteolata* Linn.	Aristolochic acid
14.	*Bauhinia acuminata* Linn.	Essential oils containing Phytol; β-Caryophyllene and Caryophyllene oxide
15.	*Begonia barbata* Wall.	Data not available to date
16.	*Begonia silhetensis* (A. DC.) C.B. Clarke	Data not available to date
17.	*Belamcanda chinensis* (L.) DC.	Iridin; Irigenin; Mangiferin; Rhamnocitrin
18.	*Breynia retusa* (Dennst.) Alston	Data not available to date
19.	*Calotropis procera* (Ait.) Ait.f.	β-Amyrin; Calactin, Coroglaucigenin and various other cardenolides; Calotroposides H-N; 2'-Epi-uscharin; Proceraside A
20.	*Cassia alata* L.	Chrysophanol; Emodin; Rhein; Aloe-emodin; Chrysophanic acid; Kaempferol; Quercetin; Myricetin
21.	*Casuarina equisetifolia* L.	Tannins; Gallic acid; various Catechol derivatives; Kaempferol; Quercetin
22.	*Centella asiatica* (L.) Urb.	α-Humulene; α-Pinene; α-Terpeneol; Asiatic acid; Asiaticoside; Astragalin; β-bisabolene; β-carotene; β-elemene; β-sesquiphellandrene; β-Sitosterol; Betulinic acid; delta-cadinene; Farnesol; Geraniol; Isoquercetin; Kaempferol; Limonene; Linalool; Madecassoside; Myrcene; Nerol; Nerolidol; Quercetin

Table 9.3 contd. ...

...Table 9.3 contd.

Serial No.	Botanical name	Notable bioactive phytochemicals
23.	*Citrus aurantifolia* (Christm.) Swingle	1,8-Cineole; 1,4-Cineole; 5-Methoxy-Psoralen; α-Phellandrene; α-Pinene; α-Terpenene, α-Terpineol; Bergamottin; Bergapten; β-Bisabolene; β-Pinene; Borneol; β-Caryophyllene; Citral; Citronellal; γ-Terpinene; Geranial; Imperatorin; Isoimperatorin; Linalool; Myrcene
24.	*Clausena suffruticosa* Wight & Arn.	Coumarins, O-Methylheptaphylline, Capnolactone
25.	*Clerodendrum indicum* (L.) Kuntze.	Scutellarein
26.	*Clerodendrum viscosum* Vent.	Quercetin; β-Sitosterol
27.	*Cocos nucifera* L.	Amyrins; α-Terpineol; β-Sitosterol; Ferulic acid; Gentisic acid; Limonene; Menthol
28.	*Curculigo recurvata* Dryand.	Data not available to date
29.	*Cuscuta reflexa* Roxb.	Bergenin; β-Sitosterol; Kaempferol; Luteolin; Mangiferin
30.	*Cyperus difformis* L.	Essential oil containing predominantly Cyperene and Cyperotundone
31.	*Delonix regia* (Bojer) Raf.	Tannins
32.	*Dillenia indica* L.	Betulin; Betulinic Acid
33.	*Dioscorea glabra* Roxb.	Ferulic acid
34.	*Dioscorea pentaphylla* L.	Diosbulbin B
35.	*Dioscorea* sp.	Various sesquiterpenoids, polysaccharides
36.	*Elephantopus scaber* L.	Deoxyelephantopin; Isodeoxyelephantopin; Lupeol
37.	*Erythrina variegata* L.	β-Sitosterol; Erysodine; Erysovine; Erysotrine; Erythraline; Oxyresveratrol
38.	*Eupatorium odoratum* L.	β-Amyrin; Lupeol; Chlorogenic acid; Hexacosanol; Odoratin
39.	*Euphorbia hirta* L.	α-Amyrin; β-Amyrin; β-Sitosterol; Betulin; Caffeic acid; Ellagic acid; Ferulic acid; Gallic acid; Kaempferol; p-Coumaric acid; Quercetin; Rhamnetin; Tannic acid
40.	*Globba marantina* L.	Essential oil containing β-Caryophyllene; α-Humulene; (Z)-Nerolidol; (Z,Z)-Farnesol
41.	*Gymnopetalum cochinchinense* (Lour.) Kurz.	Data not available to date
42.	*Hedyotis scandens* Roxb.	Phenolic glycosides
43.	*FHoya parasitica* Wall.	Dihydrocanaric acid
44.	*Hyptis suaveolens* (L.) Poit.	1,8-Cineole; α-Cadinol; α-Humelene; α-Phellandrene; α-Pinene; α-Terpinene; α-Terpineol; β-Pinene; Betulinic acid; Borneol; Camphene; Camphor; Caryophyllene oxide; Elemene; Fenchone; γ-Terpinene; Limonene; Menthol; Myrcene; p-Cymene; Terpinen-4-ol; Terpinolene; Thymol
45.	*Ixora parviflora* Vahl	β-Sitosterol; β-Sitosterol-β-D-glucoside; Kaempferol; Kaempferol-7-O-Me ether; Chlorogenic acid; Apigenin; Quercetin; Apigenin-7-O-β-D-glucopyranoside; Quercetin-3-O-β-D-galactopyranoside
46.	*Kalanchoe pinnata* (Lam.) Pers.	α-Amyrin; β-Amyrin; β-Sitosterol; Caffeic acid; Ferulic acid; Friedelin; Kaempferol; p-Coumaric acid; Patuletin; Quercetin
47.	*Lygodium flexuosum* (L.) Sw.	Kaempferol; Lygodinolide; Quercetin
48.	*Mikania cordata* (Burm.f.) B.L. Rob.	Deoxymikanolide
49.	*Mimosa pudica* L.	Mimosine; β-Amyrin; β-Sitosterol

Table 9.3 contd....

...Table 9.3 contd.

Serial No.	Botanical name	Notable bioactive phytochemicals
50.	*Moghania macrophylla* (Willd.) Kuntze	Genistein; 2-Hydroxy Genistein
51.	*Morinda angustifolia* Roxb.	1,8-dihydroxy-2-methyl-3,7-dimethoxyanthraquinone
52.	*Mucuna gigantean* (Willd.) DC.	Data not available to date
53.	*Mussaenda corymbosa* Roxb.	Data not available to date
54.	*Nymphaea nouchali* Burm.f.	Gallic acid; Myricitrin; Nymphalin
55.	*Ocimum americanum* L.	Betulinic acid; Estragole; Linalool; Cineole; Camphor; Eugenol; Ursolic acid; Rosmarinic acid
56.	*Opuntia dillenii* Ker-Gawl.	Opuntiol; Opuntioside; Polysaccharides
57.	*Phyllanthus emblica* L.	β-Sitosterol; Corilagin; Ellagic acid; Gallic acid; Gallic acid ethyl ester; Kaempferol; Lupeol; Quercetin; Rutin
58.	*Premna scandens* Roxb.	Data not available to date
59.	*Psychotria calocarpa* Kurz.	Data not available to date
60.	*Sansevieria roxburghiana* Schult. and Schult.f.	Protocatechuic acid
61.	*Sarcochlamys pulcherrima* (Roxb.) Gaud.	Data not available to date
62.	*Scoparia dulcis* L.	Betulinic acid; Coixol; Friedelin
63.	*Selaginella decipiens* Warb.	Data not available to date
64.	*Smilax macrophylla* Willd.	Data not available to date
65.	*Solanum lasiocarpum* Dunal	Data not available to date
66.	*Solanum torvum* Sw.	Methyl caffeate; some steroidal glycosides such as Torvpregnanosides A and B; Torvanol A
67.	*Spilanthes calva* DC.	
68.	*Spondias pinnata* Kurz	Gallic acid; Methyl gallate
69.	*Spondias cytherea* Sonn.	Data not available to date
70.	*Synedrella nodiflora* (L.) Gaertn.	Data not available to date
71.	*Syzygium cumini* (L.) Skeels	Betulinic acid; Gallic acid; 1,8-Cineole; Acetyl oleanolic acid; Bergenins; Caffeic acid; Cinnamaldehyde; Citronellol; Cyanidin diglycoside; Delphinidin-3-gentiobioside; Ellagic acid; Eugenol; Ferulic acid; Isoquercetin; Kaempferol; Linalool; Malvidin and malvidin derivatives; Myricetin and myricetin derivatives; Myrtenol; Nerol; N-hentriacontane; Petunidin; Quercetin; Terpinolene; α-Terpenol; α-Terpinene; β-Phellandrene; β-Pinene; β-Sitosterol
72.	*Tabernaemontana divaricata* (L.) R. Br. Roxb.	Coronaridine; Conophylline; 3'-R/S-hydroxyvoacamine; Conolidine; 19,20-dihydrotabernamine; 19,20-dihydroervahanine A.
73.	*Tamarindus indica* L.	(–) Epicatechin; α-Humulene; α-Pinene; α-Terpineol; β-Elemene; β-Ionone; β-Pinene; β-Sitosterol; Carvacrol; Cinnamaldehyde; Geranial; Geraniol; Hordenine; Isoorientin; Isovitexin; Limonene; Linalool; Methyl salicylate; Myrcene; Myristic acid; Nerol; Piperitone; Safrole; Terpinen-4-ol; Vitexin
74.	*Terminalia bellirica* (Gaertn.) Roxb.	β-Sitosterol; Corilagin; Ellagic acid; Ethyl gallate; Gallic acid; Phyllemblin
75.	*Terminalia chebula* Retz.	β-Sitosterol; Chebulagic acid; Chebulinic acid; Corilagin; Daucosterol; Ellagic acid; Gallic acid; p-Coumaric acid; Punicalagin; Punicalin; Quercetin; Rutin; Terchebin

Table 9.3 contd....

...Table 9.3 contd.

Serial No.	Botanical name	Notable bioactive phytochemicals
76.	*Thunbergia grandiflora* Roxb.	Iridoid glycosides - Isoundedoside and Grandifloric acid Apigenin 7-glucuronide
77.	*Thysanolaena maxima* (Roxb.) Kuntze	Data not available to date
78.	*Trichosanthes tricuspidata* Lour.	Trichoterol; Tricuspidatin; 2-O-glucocucurbitacin; Methyl palmitate; Palmitic acid; Suberic acid; α-Spinasterol; α-Spinasterol 3-O-β-D-glucopyranoside; Stigmast-7-en-3-β-ol-3-O-β-D-glucopyranoside; Glyceryl-1-palmitate; Glyceryl-1-stearate; Bryonolic acid; Cucurbitacin B; Isocucurbitacin B; 3-epi-Isocucurbitacin B; 23,24-Dihydrocucurbitacin D; Isocucurbitacin D; Cyclotricuspidosides A, B and C
79.	*Urena lobata* L.	Clematoside-S
80.	*Vitex negundo* L.	β-Sitosterol; β-Sitosterol acetate; Luteolin; Vitexin and its glycosides; Lyoniresinol; Chrysoplentin; Negundoside; Negundin B; Betulinic acid; Ursolic acid
81.	*Vitis pedata* L.	Data not available to date
82.	*Vitis pentagona* (Roxb.) Lawson	Data not available to date
83.	*Zingiber officinale* Roscoe	6-Gingerol; 10-Gingerol; 8-Gingerol; 8-Shogaol; 6-Shogaol; α-Curcumene; Borneol; 1,8-Cineole; 4-Terpineol; 6-dehydrogingerdione; α-Cadinol; α-Curumene; α-Phellandrene; α-Pinene; α-Terpinene; α-Terpineol; α-Zingiberene; β-Bisabolene; β-Elemene; β-Eudesmol; β-Ionone; β-Phellandrene; β-Pinene; β-Sesquiphellandrene; β-Sitosterol; Borneol; Bornyl acetate; Caffeic acid; Camphene; Camphor; Capsaicin; Caryophyllene; Chlorogenic acid; Citral; Citronellal; Citronellol; Curcumin; Eugenol; Farnesol; Ferulic acid; Galanolactone; Geranial; Geraniol; Isoeugenol; Kaempferol; Limonene; Linalool; Myrcene; Myricetin; Myristic acid; Nerol; Nerolidol; p-Coumaric acid; p-Cymene; Quercetin; Zingerone
84.	*Ziziphus mauritiana* Lam.	Betulin; Betulinic acid; Mauritine L; Mauritine M; Nummularines H, B; Hemsine A

[Sources: https://phytochem.nal.usda.gov; http://www.mpbd.info; PubMed (Fibers, amino acids, metal ions, vitamins, fatty acids ignored); https://scifinder.cas.org. Blank ones represent no available information.]

(Boz 2015); furthermore, it is reported to be hepatoprotective and renoprotective (Akdemir et al. 2017). The compound has also been shown to induce apoptosis in HCT-15 colon cancer cells (Jaganathan et al. 2013).

***Ocimum americanum* L.** Rosmarinic acid reportedly has antioxidant, anti-inflammatory, anti-viral, photoprotective, anti-cancer, anti-depressant, and neuroprotective actions (Bhatt et al. 2013). Eugenol is antioxidant, anti-microbial, anti-cancer, and anti-inflammatory (Raja et al. 2015).

***Phyllanthus emblica* L.** Corilagin is known to be antioxidant, radioprotective and anti-viral (Sarin et al. 2014); the compound has been reported to inhibit ovarian cancer (Jia et al. 2013). Corilagin also demonstrated anti-inflammatory and antioxidative effects in rat model of acute cholestasis (Jin et al. 2013).

***Sansevieria roxburghiana* Schult. and Schult.f.** Protocatechuic acid has been reported to have anti-inflammatory, antioxidant, anti-hyperglycemia, anti-bacterial, anti-viral, anti-cancer, anti-aging, anti-atherosclerotic, anti-tumoral, anti-asthma, anti-ulcer, anti-spasmodic and neurological properties (Khan et al. 2015).

***Scoparia dulcis* L.** Friedelin has been described possessing anti-mycobacterial activity (Mann et al. 2011); anti-inflammatory, analgesic, and anti-pyretic effects have also been described for this compound (Antonisamy et al. 2011).

***Syzygium cumini* (L.) Skeels.** Bergenins are antinociceptive, anti-arrhythmic, antioxidative, anti-microbial, hepatoprotective, anti-inflammatory,providing protection against gastric ulcers, they also possess insulin enhancing,lipolytic, and enhances wound healing properties. Caffeic acid has anti-aging, anti-atherogenic, anti-carcinogenic, anti-depressant, anti-edemic, anti-hepatoadenomic, anti-hepatotoxic, anti-inflammatory, anti-tumor, and vulnerary (wound-healing) properties. Cinnamaldehyde is an anti-hyperuricemic, acaricidal, anti-microbial, and anti-diabetic agent. Citronellol has demonstrated anti-bacterial, anti-viral, anti-spasmodic, and antioxidant activities. Eugenol is an acaricide, anti-convulsant, anti-edemic, anti-inflammatory, anti-mitotic, anti-mutagenic, antioxidant, anti-tumor, anti-bacterial, hepatoprotective, and insecticidal. Malvidin has anti-neoplastic activities. Myrtenol is anti-insomnia, anti-malarial, antioxidant, anti-plasmodial, anti-thyreotropic, aphrodisiac, gonadotrophic, hypocholesterolemic and immunostimulant. Nerol is a sedative (Ramya et al. 2012).

***Tamarindus indica* L.** β-Pinene possesses anti-inflammatory, antiseptic, candidicide, and insecticidal activities (Ramya et al. 2012). Carvacrol is an antioxidant, anti-bacterial, anti-fungal, anti-cancer and anti-inflammatory agent; it also possesses hepatoprotective, spasmolytic, and vasorelaxant properties (Suntres et al. 2015). Geraniol is an anti-hyperglycemic (Babukumar et al. 2017). Isovitexin is an anti-inflammatory and antioxidant (Lv et al. 2016). Vitexin has shown anti-inflammatory, anti-oxidant, cardioprotective, anti-cancer, antinociceptive, anti-convulsant, memory enhancer, and anti-diabetic properties (Aslam et al. 2015).

***Terminalia chebula* Retz.** Chebulagic acid is anti-hyperglycemic (Huang et al. 2012). Punicalagin(s) can be antioxidants, anti-cancer and have beneficial effects in cardiovascular disorders (Tyagi et al. 2012).

***Trichosanthes tricuspidata* Lour.** Tricuspidation may be a useful anti-cancer agent (Dhanabal et al. 2015). Spinasterol has analgesic properties (Brusco et al. 2017). Cucurbitacin B reportedly has anti-microbial, anti-cancer and anti-inflammatory properties (Chung et al. 2015).

***Vitex negundo* L.** Lyoniresinol has strong antioxidant properties (Govind 2011). Negundoside is hepatoprotective (Sheikh et al. 2008).

***Zingiber officinale* Roscoe.** Gingerols, shogaols, and zingerone have antioxidant, anti-tumor, anti-inflammatory, analgesic, anti-microbial, and hepatoprotective activities (Rahmani et al. 2014). Borneol is antinociceptive and anti-inflammatory (da Silva Almeida et al. 2013). α-Cadinol has insecticidal properties against yellow fever mosquito larvae, anti-termitic activity, anti-fungal activity against *Candida versicolor* and *Laetiporus sulphureus*, and was selectively cytotoxic against human colon adenocarcinoma (Leandro et al. 2012). Eugenol, α-pinene and β-pinene are known to inhibit growth of potential infectious endocarditis causing Gram-positive bacteria (Leite et al. 2007). Caryophyllene can be a good antioxidant (Miguel 2010).

Conclusion

To conclude, the medicinal plants of the Khyang tribe contain multiple natural product metabolites that are known to possess health-promoting, protective and disease-fighting properties. These agents, over millennia have evolved diverse structural scaffolds, which may prove invaluable in drug discovery programs. It can readily be seen from Table 9.3, that many of the Khyang medicinal plants are as yet uncharacterized and putative bioactive components await discovery. Thus, these plants may potentially yield novel therapeutic components. It is also important to point out that because of the twin effects of globalization and encroachment on forest lands pose real threats, not only are habitats of these medicinal plants shrinking but also traditional medicinal practices and the knowledge of generation of Khyangs are at risk of being lost. It is therefore important to pay attention to the conservancy status of these medicinal plants.

References

Akdemir, F.N.E., Albayrak, M., Çalik, M., Bayir, Y. and Gülçin, I. 2017. The protective effects of p-coumaric acid on acute liver and kidney damages induced by cisplatin. Biomedicines 5: 18.

Akter, S., Nipu, A.H., Chyti, H.N., Das, P.R., Islam, M.T. and Rahmatullah, M. 2014. Ethnomedicinal plants of the Shing tribe of Moulvibazar district, Bangladesh. World J. Pharm. Pharmaceut. Sci. 3(10): 1529–1537.

Al-Dhabi, N.A., Arasu, M.V., Park, C.H. and Park, S.U. 2015. An up-to-date review of rutin and its biological and pharmacological activities. EXCLI Journal 14: 59–63.

Alfarra, H.Y. and Omar, M.N. 2013. *Centella asiatica*: from folk remedy to the medicinal biotechnology—a state revision. Int. J. Biosci. 3(6): 49–67.

Antonisamy, P., Duraipandiyan, V. and Ignacimuthu, S. 2011. Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. in mouse and rat models. J. Pharm. Pharmacol. 63(8): 1070–1077.

Aprotosoaie, A.C., Hăncianu, M., Costache, I.-I. and Miron, A. 2014. Linalool: a review on a key odorant molecule with valuable biological properties. Flavour Frag. J. 29(4): 193–219.

Aslam, M.S., Ahmad, M.S. and Mamat, A.S. 2015. Pharmacological potential of vitexin. Ind. Res. J. Pharm. Sci. 5: 114–122.

Azad, A.K., Mahmud, M.R., Parvin, A., Chakrabortty, A., Akter, F., Moury, S.I., Anny, I.P., Tarannom, S.R., Joy, S.K., Chowdhury, S.Y., Akter, S. and Rahmatullah, M. 2014. Medicinal plants of a Santal tribal healer in Dinajpur district, Bangladesh. World J. Pharm. Pharmaceut. Sci. 3(10): 1597–1606.

Babukumar, S., Vinothkumar, V., Sankaranarayanan, C. and Srinivasan, S. 2017. Geraniol, a natural monoterpene, ameliorates hyperglycemia by attenuating the key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. Pharm. Biol. 55(1): 1442–1449.

Badhani, B., Sharma, N. and Kakkar, R. 2015. Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. RSC Adv. 5: 27540–27557.

Balick, J.M. and Cox, P.A. 1996. Plants, People and Culture: the Science of Ethnobotany, Scientific American Library, New York, 228 pp.

Bayala, B., Bassole, I.H., Gnoula, C., Nebie, R., Yonli, A., Morel, L., Figueredo, G., Nikiema, J.B., Lobaccaro, J.M. and Simpore, J. 2014. Chemical composition, antioxidant, anti-inflammatory and anti-proliferative activities of essential oils of plants from Burkina Faso. PLoS One 9(3): e92122.

Bhatt, R., Mishra, N. and Bansal, P.K. 2013. Phytochemical, pharmacological and pharmacokinetics effects of rosmarinic acid. J. Pharm. Sci. Innov. 2(2): 28–34.

Brusco, I., Camponogara, C., Carvalho, F.B., Schetinger, M.R.C., Oliveira, M.S., Trevisan, G., Ferreira, J. and Oliveira, S.M. 2017. α-Spinasterol: a cyclooxygenase inhibitor and a TRPV1 antagonist presents an antinociceptive effect in clinically relevant models of pain in mice. Brit. J. Pharmacol. doi: 10.1111/bph.13992.

Boz, H. 2015. p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. Int. J. Food. Sci. Technol. 50(11): 2323–2328.

Calderón-Montaño, J.M., Burgos-Morón, E., Pérez-Guerrero, C. and López-Lázaro, M. 2011. A review on the dietary flavonoid kaempferol. Mini-Rev. Med. Chem. 11: 298–344.

Carrasco-Torres, G., Monroy-Ramírez, H.C., Martínez-Guerra, A.A., Baltiérrez-Hoyos, R., Romero-Tlalolini, M.L.Á., Villa-Treviño, S., Sánchez-Chino, X. and Vásquez-Garzón, V.R. 2017. Quercetin reverses rat liver preneoplastic lesions induced by chemical carcinogenesis. Oxid. Med. Cell. Longev. 2017: 4674918.

Chung, S.O., Kim, Y.J. and Park, S.U. 2015. An updated review of cucurbitacins and their biological and pharmacological activities. Exp. Clin. Sci. J. 14: 562–566.

Dall'Acqua, S., Peron, G., Ferrari, S., Gandin, V., Bramucci, M., Quassinti, L., Mártonfi, P. and Maggi, F. 2017. Phytochemical investigations and antiproliferative secondary metabolites from *Thymus alternans* growing in Slovakia. Pharm. Biol. 55(1): 1162–1170.

da Silva Almeida, J.R.G., Souza, G.R., Silva, J.C., de Lima Saraiva, S.R.G., de Oliveira Júnior, R.G., de Souza SiqueiraQuintans, J., de Souza SiqueiraBarreto, R., Bonjardim, L.R., de HolandaCavalcanti, S.C. and Quintans Junior, L.J. 2013. Borneol, a bicyclic monoterpene alcohol, reduces nociceptive behavior and inflammatory response in mice. Sci. World J., 2013: Article ID 808460.

Dhanabal, S.P., Lall, N., Pavithra, N. and Chaitanya, M.V.N.L. 2015. Natural products: an important leads for anticancer drug discovery. J. Harm. Res. Pharm. 4(4): 316–328.

Dong, X., Fu, J., Yin, X., Cao, S., Li, X., Huyiligeqi and Ni, J. 2016. Emodin: a review of its pharmacology, toxicity and pharmacokinetics. Phytother. Res. 30(8): 1207–1218.

El-Soud, N.H.A. 2010. Herbal medicine in ancient Egypt. J. Med. Plants Res. 4(2): 82–86.

Govind, P. 2011. Some important anticancer herbs: a review. Int. Res. J. Pharm. 2(7): 45–52.

Huang, Y.-N., Zhao, D.-D., Gao, B., Zhong, K., Zhu, R.-X., Zhang, Y., Xie, W.-J., Jia, L.-R. and Gao, H. 2012. Anti-hyperglycemic effect of chebulagic acid from the fruits of *Terminalia chebula* Retz. Int. J. Mol. Sci. 13: 6320–6333.

Islam, A., Siddik, A.B., Hanee, U., Guha, A., Zaman, F., Mokarroma, U., Zahan, H., Jabber, S., Naurin, S., Kabir, H., Jahan, S. and Rahmatullah, M. 2015a. Ethnomedicinal practices of a chakma tribal healer practicing among a marma community in Rangamati district, Bangladesh. World J. Pharm. Pharmaceut. Sci. 4(3): 180–188.

Islam, A., Siddik, A.B., Hanee, U., Guha, A., Zaman, F., Mokarroma, U., Zahan, H., Jabber, S., Naurin, S., Kabir, H., Jahan, S. and Rahmatullah, M. 2015b. Ethnomedicinal practices among a Tripura community in Rangamati district, Bangladesh. World J. Pharm. Pharmaceut. Sci. 4(3): 189–196.

Jaganathan, S.K., Supriyanto, E. and Mandal, M. 2013. Events associated with apoptotic effect of p-coumaric acid in HCT-15 colon cancer cells. World J. Gastroenterol. 19(43): 7726–7734.

Jia, L., Jin, H., Zhou, J., Chen, L., Lu, Y., Ming, Y. and Yu, Y. 2013. A potential anti-tumour herbal medicine, Corilagin, inhibits ovarian cancer cell growth through blocking the TGF-β signaling pathways. BMC Complem. Altern. M. 13: 33.

Jin, F., Cheng, D., Tao, J.-Y., Zhang, S.-L., Pang, R., Guo, Y.-J., Ye, P., Dong, J.-H. and Zhao, L. 2013. Anti-inflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis. BMC Gastroenterol. 13: 79.

Jyotshna, Khare, P. and Shanker, K. 2016. Mangiferin: A review of sources and interventions for biological activities. Biofactors 42(5): 504–514.

Kelly, G.S. 2011. Quercetin. Altern. Med. Rev. 16(2): 172–194.

Khan, A.K., Rashid, R., Fatima, N., Mahmood, S., Mir, S., Khan, S., Jabeen, N. and Murtaza, G. 2015. Pharmacological activities of protocatechuic acid. Acta Pol. Pharm. 72(4): 643–650.

Khorsandi, L., Orazizadeh, M., Niazvand, F., Abbaspour, M.R., Mansouri, E. and Khodadadi, A. 2017. Quercetin induces apoptosis and necroptosis in MCF-7 breast cancer cells. Bratisl. Med. J. 118(2): 123–128.

Leandro, L.M., de Sousa Vargas, F., Barbosa, P.C.S., Neves, J.K.O., da Silva, J.A. and da Veiga-Junior, V.F. 2012. Chemical and biological activities of terpenoids from Copaiba (*Copaifera* spp.) oleoresins. Molecules 17: 3866–3889.

Leite, A.M., de Oliveira Lima, E., de Souza, E.L., Diniz, M.F.F. deM, Trajano, V.N. and de Medeiros, I.A. 2007. Inhibitory effect of β-pinene, α-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. Braz. J. Pharm. Sci. 43(1): 121–126.

Lin, F., Zhang, C., Chen, X., Song, E., Sun, S., Chen, M., Pan, T. and Deng, X. 2015. Chrysophanol affords neuroprotection against microglial activation and free-radical-mediated oxidative damage in BV2 murine microglia. Int. J. Clin. Exp. Med. 8(3): 3447–3455.

Liu, T., Ma, H., Shi, W., Duan, J., Wang, Y., Zhang, C., Li, C., Lin, J., Li, S., Lv, J. and Lin, L. 2017. Inhibition of STAT3 signaling pathway by ursolic acid suppresses growth of hepatocellular carcinoma. Int. J. Oncol. 51(2): 555–562.

Lv, H., Yu, Z., Zheng, Y., Wang, L., Qin, X., Cheng, G. and Ci, X. 2016. Isovitexin exerts anti-inflammatory and anti-oxidant activities on lipopolysaccharide-induced acute lung injury by inhibiting MAPK and NF-κB and activating HO-1/Nrf2 pathways. Int. J. Biol. Sci. 12(1): 72–86.

Maalik, A., Bukhari, S.M., Zaidi, A., Shah, K.H. and Khan, F.A. 2016. Chlorogenic acid: A pharmacologically potent molecule. Acta Pol. Pharm. 73(4): 851–854.

Magnani, C., Isaac, V.L.B., Correa, M.A. and Salgado, H.R.N. 2014. Caffeic acid: a review of its potential use in medications and cosmetics. Anal. Methods 6: 3203–3210.

Mann, A., Ibrahim, K., Oyewale, A.O., Amupitan, J.O., Fatope, M.O. and Okogun, J.I. 2011. Antimycobacterialfriedelane-terpenoid from the root bark of *Terminalia avicennioides*. Am. J. Chem. 1(2): 52–55.

Martin, G.J. 1995. Ethnobotany: a 'People and Plants' Conservation Manual, Chapman and Hall, London, pp. 268.

Maundu, P. 1995. Methodology for collecting and sharing indigenous knowledge: a case study. Indigen. Know. Developm. Mon. 3(2): 3–5.

Miguel, M.G. 2010. Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules 15: 9252–9287.

Moreira, J., Klein-Júnior, L.C., Filho, V.C. and de Campos Buzzi, F. 2013. Anti-hyperalgesic activity of corilagin, a tannin isolated from *Phyllanthus niruri* L. (Euphorbiaceae). J. Ethnopharmacol. 146(1): 318–323.

Mythili Avadhani, M.N., Immanuel Selvaraj, C., Rajasekharan, P.E. and Tharachand, C. 2013. The sweetness and bitterness of sweet flag [*Acorus calamus* L.]—a review. Res. J. Pharm. Biol. Chem. Sci. 4(2): 598–610.

Nabavi, S.F., Braidy, N., Gortzi, O., Sobarzo-Sanchez, E., Daglia, M., Skalicka-Woźniak, K. and Nabavi, S.M. 2015. Luteolin as an anti-inflammatory and neuroprotective agent: a brief review. Brain Res. Bull. 119: 1–11.

Nayeem, N., Asdaq, S.M.B., Salem, H. and Said, A.H.E.I.-A. 2016. Gallic acid: a promising lead molecule for drug development. J. Appl. Pharm. 8: 213.

Neta, M.C.S., Vittorazzi, C., Guimarães, A.C., Martins, J.D.L., Fronza, M., Endringer, D.C. and Scherer, R. 2016. Effects of β-caryophyllene and *Murraya paniculata* essential oils in the murine hepatoma cells and in the bacteria and fungi 24-h time-kill curve studies. Pharm. Biol. 55(1): 190–197.

Ong, K.C. and Khoo, H.E. 1997. Biological effects of myricetin. Gen. Pharmacol. 29(2): 121–126.

Ou, S. and Kwok, K.-C. 2004. Ferulic acid: pharmaceutical functions, preparation and applications in foods. J. Sci. Food Agric. 84: 1261–1269.

Pandurangan, A.K. and Esa, N.M. 2014. Luteolin, a bioflavonoid inhibits colorectal cancer through modulation of multiple signaling pathways: a review. Asian Pac. J. Cancer Prev. 15: 5501–5508.

Pollier, J. and Goossens, A. 2012. Oleanolic acid. Phytochemistry 77: 10–15.

Rahman, F. and Rahmatullah, M. 2015. Medicinal plant formulations of the Musohor tribe of Birganj in Dinajpur district, Bangladesh. World J. Pharm. Pharmaceut. Sci. 4(11): 177–185.

Rahmani, A.H., Shabrmi, F.M.A. and Aly, S.M. 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int. J. Physiol. Pathophys. Pharmacol. 6(2): 125–136.

Raja, M.R.C., Srinivasan, V., Selvaraj, S. and Mahapatra, S.K. 2015. Versatile and synergistic potential of eugenol: a review. Pharm. Anal. Acta 6(5): 367.

Ramya, S., Neethirajan, K. and Jayakumararaj, R. 2012. Profile of bioactive compounds in *Syzygium cumini*—a review. J. Pharm. Res. 5(8): 4548–4553.

Saad, B., Azaizeh, H. and Said, O. 2005. Tradition and perspectives of Arab herbal medicine: a review. Evid.-Based Compl. Alt. 2: 475–479.

Saeidnia, S., Manayi, A., Gohari, A.R. and Abdollahi, M. 2014. The story of beta-sitosterol—a review. Eur. J. Med. Plant. 4(5): 590–609.

Santos, F.A., J.T. Frota, B.R. Arruda, T.S. de Melo, A.A. de Carvalho Almeida da Silva, G.A. de Castro Brito, M.H. Chaves and V.S. Rao. 2012. Antihyperglycemic and hypolipidemic effects of α,β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. Lipids Health Dis. 11: 98.

Sarin, B., Verma, N., Martín, J.P. and Mohanty, A. 2014. An overview of important ethnomedicinal herbs of *Phyllanthus* species: Present status and future prospects. Sci. World J. 2014: Article ID 839172.

Seraj, S., Mazumder, M.S.H., Hossan, M.S. and Rahmatullah, M. 2017. Uncommon phytotherapeutic practices in Hajiganj Upazila of Chandpur District, Bangladesh against gastrointestinal disorders. EC Gastroenterol. Dig. Syst. 4(3): 68–74.

Sheikh, A.T., Kaiser, P.J., Gupta, B.D., Gupta, V.K. and Johri, R.K. 2008. Negundoside, an iridiod glycoside from leaves of *Vitex negundo*, protects human liver cells against calcium mediated toxicity induced by carbon tetrachloride. World J. Gastroenterol. 14(23): 3693–3709.

Sofowora, A. 1982. Medicinal Plants and Traditional Medicinal in Africa. John Wiley and Sons, New York, 256 pp.

Sonoda, J.-I., Narumi, K., Akio, K., Erisa, T. and Toshiro, M. 2015. Green tea catechins—Pharmacokinetic properties and health beneficial effects. Pharm. Anal. Acta. 6: 2.

Starzengruber, P., Fuehrer, H.P., Ley, B., Thriemer, K., Swoboda, P., Habler, V.E., Jung, M., Graninger, W., Khan, W.A., Haque, R. and Noedl, H. 2014. High prevalence of asymptomatic malaria in south-eastern Bangladesh. Malaria J. 13: 16.

Subbarayappa, B.V. 2001. The roots of ancient medicine: an historical outline. J. Biosci. 26(2): 135–144.

Suntres, Z.E., Coccimiglio, J. and Alipour, M. 2015. The bioactivity and toxicological actions of carvacrol. Crit. Rev. Food Sci. 55: 304–318.

Tyagi, S., Singh, A., Bhardwaj, P., Sahu, S., Yadav, A.P. and Kori, M.L. 2012. Punicalagins—a large polyphenol compounds found in pomegranates: a therapeutic review. Acad. J. Plant Sci. 5(2): 45–49.

Varga, Z.V., Matyas, C., Erdelyi, K., Cinar, R., Nieri, D., Chicca, A., Nemeth, B.T., Paloczi, J., Lajtos, T., Corey, L., Hasko, G., Gao, B., Kunos, G., Gertsch, J. and Pacher, P. 2017. β-Caryophyllene protects against alcoholic steatohepatitis by attenuating inflammation and metabolic dysregulation in mice. Brit. J. Pharmacol. doi:10.1111/bph.13722.

Vattem, D.A. and Shetty, K. 2005. Biological functionality of ellagic acid: a review. J. Food Biochem. 29(3): 234–266.

Vishnu Varthan, V.J., Srividya, A.R. and Sathish Kumar, M.N. 2013. Int. J. Pharm. Res. Schol. 2(4): 198–212.

Wal, P., Wal, A., Sharma, G. and Rai, A.K. 2011. Biological activities of lupeol. Sys. Rev. Pharm. 2(2): 96–103.

Xu, L., Liu, C., Xiang, W., Chen, H., Qin, X. and Huang, X. 2014. Advances in the study of oxyresveratrol. Int. J. Pharmacol. 10(1): 44–54.

Yang, H.-Y., Chen, P.-C. and Wang, J.-D. 2014. Chinese herbs containing aristolochic acid associated with renal failure and urothelial carcinoma: A review from epidemiologic observations to casual inference. BioMed Res. Int. 2014: Article ID 569325.

Zaman, T., Keya, K.F., Akter, S., Sagar, M.H., Khan, M.S., Bhuiyan, M.B., Malek, I. and Rahmatullah, M. 2015. Plants as Medicines: Documentation of medicinal plants used by a Khasia tribal practitioner in Habiganj district, Bangladesh. World J. Pharm. Pharmaceut. Sci. 4(12): 55–63.

Zhao, R., Li, T., Zheng, G., Jiang, K., Fan, L. and Shao, J. 2017. Simultaneous inhibition of growth and metastasis of hepatocellular carcinoma by co-delivery of ursolic acid and sorafenib using lactobionic acid modified and pH-sensitive chitosan-conjugated mesoporous silica nanocomplex. Biomaterials 143: 1–16.

10

Management of Cardiovascular Diseases and Related Complications Using Traditional Herbs and Spices

Kavisha Mooroteea and *Fawzi Mahomoodally**

Introduction

The World Health Organization (WHO) defines cardiovascular diseases (CVDs) as chronic disorders that affect the cardiovascular system, which consist of the heart and blood vessels running throughout the body (WHO 2017). CVDs are the leading cause of mortality worldwide (WHO 2016) responsible for around 17.7 million deaths in 2015 and it is predicted that by 2030, the death toll will escalate up to 23.3 million (WHO 2013, WHO 2017).

Estimated direct and indirect costs of CVDs in the United States are greater than US$286 billion. Cardiovascular diseases impose direct costs including hospital and nursing home services, professional fees, prescription drugs, and transportation costs spent to visit healthcare providers and indirect costs including costs incurred because of disability and loss of productivity due to absence from work (Roger and colleagues 2011).

Some of the common types of CVDs and related complications include ischaemic heart disease, cerebrovascular disease, chronic rheumatic heart disease, hypertensive disease, hyperlipidemia, pulmonary heart disease, diseases of the blood vessels, and other forms of heart diseases (WHO 2016).

Ischaemic Heart Disease (IHD) also called Coronary Heart Disease (CHD) is caused when the coronary arteries present on the surface of the heart are narrowed and blocked due to fatty material deposition. As a result, the heart muscle does not get enough blood and oxygen supply to meet its metabolic demands. IHD can thus lead to angina pectoris, heart attack, and heart failure (Thygesen et al. 2007). IHD was found to be the leading global cause of mortality and life-years lost in 2010 (Murray et al. 2012).

The WHO definition of cerebrovascular disease generally termed as stroke is a clinical syndrome characterized by rapidly developing signs of focal or global disturbance of cerebral functions lasting longer than 24 hours or resulting in mortality with no apparent causes other that of vascular origin (Eleftheriou 2012).

Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Mauritius.
* Corresponding author: f.mahomoodally@uom.ac.mu

Stroke is the third leading cause of mortality worldwide responsible for more than 5 million deaths annually (Eleftheriou 2012).

The two major types of stroke are:

- Ischemic stroke which occurs when a blood vessel (artery) carrying blood to the brain is blocked due to atherosclerosis preventing blood from reaching the brain and the brain tissue dies due to lack of oxygen (Torbey and Bhardwaj 2004).
- Brain haemorrhage which occurs when a brain artery bursts causing blood to leak into the brain tissue (Dupont et al. 2010).

Chronic Rheumatic Heart Disease (RHD) is a common form of heart disease caused by infection. Group A *Streptococcus* bacteria infects the throat which leads to Acute Rheumatic Fever (ARF) and finally to RHD. Chronic RHD permanently affects the heart valves of a person and can lead to heart failure, sudden cardiac death, atrial fibrillation, and embolic stroke in the future (Mackay et al. 2004, Khan and Mensah 2010). According to WHO, RHD is responsible for around 1.5% of deaths yearly (WHO Department of Child and Adolescent Health and Development 2005, Khan and Mensah 2010).

Hypertension also called high Blood Pressure (BP) is a chronic disorder in which the BP in the arteries is above normal (Tabassum and Ahmad 2011). It affects more than 1 billion people globally (Chobanian et al. 2003).

Two main types of hypertension are known which include:

- Essential hypertension: It is the most common one affecting 90 to 95% of hypertensive patients without a known cause but there are many factors such as nutrition, age, lifestyle, neurohumoral activity, and interactions that increase the risk of acquiring essential hypertension (Tabassum and Ahmad 2011).
- Secondary hypertension: It affects 5–10% of hypertensive patients and has a well-established cause such as diabetes and renal damage and therefore is more likely to be managed compared to essential hypertension (Tabassum and Ahmad 2011).

Hyperlipidemia refers to raised blood lipid concentration. Elevation in Low Density Lipoproteins (LDL), cholesterol (esters derivatives) and triglycerides are primarily responsible for hyperlipidemia. These lipids are coupled with blood plasma proteins and remain in the dissolved state in the blood. The main reason for hyperlipidemia is faulty lipid metabolism which is caused by the defect in the activity of lipoprotein lipase enzyme or lack of the surface Apoprotein C-II. Different types of hyperlipidemia exist depending on which lipid levels are high in the blood (Nirosha et al. 2014). For instance, hypercholesterolemia results when there is a high level of cholesterol, whereas hypertriglyceridemia results when there is a high amount of triglycerides, the most common form of fat.

The main focus of this chapter is to provide an overview of common cardiovascular diseases and to highlight the potential of traditional medicines, particularly herbs and spices, in the management of CVDs and related complications.

Risk factors associated with cardiovascular diseases

Various risk factors contribute to developing CVDs and these risk factors may be classified as either non modifiable or modifiable. Non modifiable risk factors are conditions that cannot be changed whereas modifiable risk factors are conditions that can be altered through lifestyle changes (Buttar et al. 2005).

- **Non modifiable risk factors**
  Age, heredity or genetic makeup, and type 1 diabetes are classified as non-modifiable risk factors. Age is a process of wear and tear the body undergoes with time making a person more susceptible to chronic diseases such as CVDs. Further ageing makes the body exposed to multiple stressors and oxidative stress which cause harm to the body. According to epidemiological research, people with a family history of heart disease are more vulnerable to develop CVDs. Moreover, a type 1 diabetic has abnormal fat metabolism and impaired glucose tolerance increasing the risk of developing CVDs (Buttar et al. 2005).

- **Modifiable risk factors**
  Nine major modifiable risk factors for CVDs which are tobacco smoking, excessive alcohol, lack of physical activity, poor nutrition, obesity, hypertension, high amount of dietary fat, and high blood sugar level were identified by the Canadian Heart and Stroke Foundation in 2003 (Heart and Stroke Foundation 2005, cited Buttar et al. 2005). Also, sudden stress, recurrent migraine, and the use of oral contraceptives were linked with CVDs (Buttar et al. 2005).

Traditional medicine

The WHO stated that Traditional Medicine (TM) refers to health practices, approaches, knowledge, and beliefs encompassing animal and mineral based medicines, spiritual therapies, manual techniques, and exercises applied singularly or in amalgamation to cure, diagnose, and prevent diseases or to restore good health (WHO 2003).

Herbal medicines have subsisted throughout the world with a prolonged recorded history since the prehistoric period. Herbs were used in ancient Chinese, Greek, Egyptian, and Indian medicine for several remedial purposes. However, the Native Americans and Africans use them in their healing rituals as a part of their culture. The traditional Indian system has incorporated herbs as one of its most potent therapeutic components, which are documented in the literature such as Vedas and Samhitas (Ehrlich 2011).

In the early 19th century, scientists had access to chemical analysis methods and thus they isolated and modified active constituents from the herbs, resulting in transition from raw herbals to artificial pharmaceuticals. During that phase, there was a fall in the use of medicinal herbs (Ehrlich 2011). However, artificial pharmaceuticals were relatively more costly and generated many unwanted side effects despite their powerful pharmacological activity. This is why nowadays both consumers and the scientific community are shifting back to herbal medicines, which are derived from the nature and claim to be safer (Oreagba et al. 2011).

Use of traditional medicine worldwide

Since the beginning of mankind, TM largely medicinal herbs and spices have been used to manage ailments. Undoubtedly, they are still being utilized for prophylactic and curative purposes all throughout the world. TM has sustained its popularity in all areas of the developing world. According to WHO, 70% of Indians rely on TM for their primary health care needs. In China, TM accounts for about 40% of all health care provided and more than 90% of general hospitals in China have units for TM (WHO 2005, Fig. 10.1).

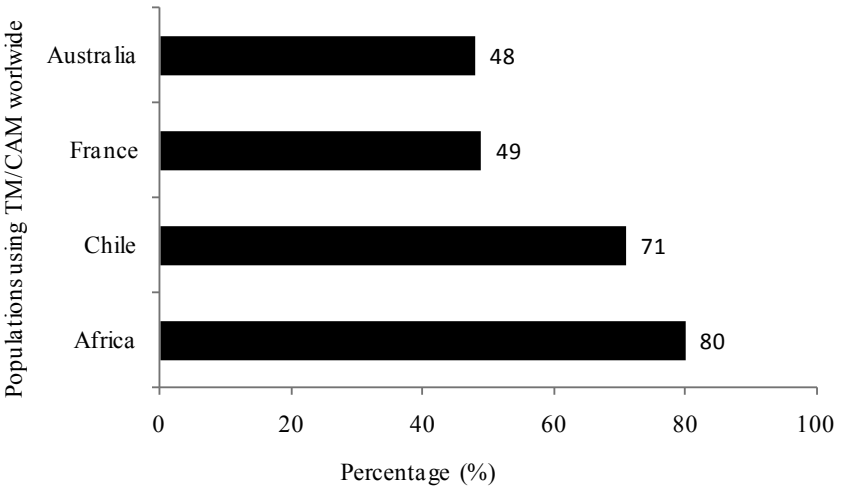


Fig. 10.1 Populations using TM/CAM worldwide.

Nonetheless, use of TM is not limited to developing countries only and its use is rapidly spreading in developed countries. In the United States, 158 million adults depend on natural remedies and according to the USA Commission for Alternative and Complementary medicines, US $17 billion were expended on TM in 2000 (WHO 2003).

Factors influencing use of traditional medicine

There is no denying to the clear-cut fact that use of TM is growing worldwide and public interest in traditional remedies has been ascribed to various factors including:

- lower cost and fewer side effects compared to allopathic drugs
- strong perception on the safety and efficacy of TM
- burden of diseases and failure of conventional treatment
- family tradition or culture
- regional biodiversity

Systems of traditional medicine

Throughout history, a variety of TM systems have been developed and the prevailing conditions, environment, and geographic area affect the philosophy and practices of each system (WHO 2005). The main systems of TM include Traditional Chinese Medicine (TCM), traditional Indian medicine, Traditional Arabic Medicine (TAM), and traditional African medicine (TAM).

- **Traditional Chinese medicine**
  Traditional Chinese medicine originated from China and has a history of several thousands of years (Xutian et al. 2009, Wachtel-Galor and Benzie 2011). Yin and Yang is an important theory in TCM as it is the foundation of diagnosis and treatment which are based on a holistic view. TCM incorporates a range of practices such as herbal medicines (*Ginkgo biloba, Allium sativum*, and *Panax ginseng*), acupuncture, moxibustion, mind/body exercises, and massage which are being used worldwide for supporting good health and for prophylaxis of ailments (Wachtel-Galor and Benzie 2011).

- **Traditional Indian medicine**
  Traditional Indian medicine also known as Ayurveda originated years ago in India (Prasad, 2002) and is a unique and distinct health care system due to its universal and holistic approach. This system emphasizes on living in harmony with the Universe which constitutes five basic elements namely Earth, Water, Fire, Air, and Space. Along with these five elements, Ayurveda also uses herbs (*Curcuma longa, Magnifera indica,* and *Phyllanthus emblica*), massage, diet, yoga, and detoxification for sustaining physical, psychological, philosophical, ethical, and spiritual well-being of mankind and maintaining good health (Kurup 2004, Ravishankar and Shukla 2007).

- **Traditional Arabic medicine**
  Traditional Arabic medicine also known as Unani medicine or Greco-Arab medicine originated in Greece and was developed by Arabs and Persians into an elaborate medical science. Unani system of medicine aims at treating the whole body, the mind, and the soul. This system is based on hippocratic theory of four humors: blood, phlegm, yellow bile, and black bile which have different temperaments: hot, cold, wet, and dry (Rahman et al. 2008). In Unani, techniques such as regimental therapy (Ilaj-bil-Tadbeer), diet therapy (Ilaj-bil-Ghiza), massage (Dalak), pharmacotherapy (Ilaj-bid-Dawa), and surgery (Jarahat) are used for health rehabilitation (Ahmed et al. 2014).

- **Traditional African medicine**
  Traditional African medicine is the most ancient and maybe the most varied of all medicinal systems. Africa is regarded as the cradle of mankind with a substantial natural and cultural diversity marked by regional differences in curative practices (Gurib-Fakim 2006). African traditional medicine in its diverse forms is holistic consisting of the body and the mind. The traditional healer generally

identifies and treats the psychological basis of a disease prior to prescribing medicines to cure the symptoms (Gurib-Fakim 2006, Gurib-Fakim et al. 2010).

According to Mahomoodally (2013), two significant reasons advocate the continuous enthusiasm in TM in the African healthcare system, primarily:

1. Deficient access to conventional prescriptions and western types of medications, whereby access to modern medical care cannot be afforded by most people in Africa either in light of the fact that it is too expensive or on the grounds that there are no medical service providers.
2. Absence of powerful modern medical treatment for some sicknesses such as, malaria and/or HIV/AIDS, which inordinately influence Africa more than other areas in the world although worldwide in distribution.

Some of the commonly used African medicinal plants include *Acacia senegal, Aloe ferox, Artemisia herba-alba, Aspalathus linearis, Centella asiatica, Catharanthus roseus, Cyclopia genistoides, Harpagophytum procumbens, Momordica charantia,* and *Pelargonium sidoides.*

Regulation of herbal medicines

Herbal medicines are normally sold as food supplements, but there is a lack of a standard regulatory framework in different countries thereby impeding the international trade and expansion of the herbal products segment. The present law in United States, Canada, and Europe could be used to direct the legal features of the herbal medicine industry in other countries (Benzie and Wachtel-Galor 2011).

In the United States, under the Dietary Supplement Health and Education Act (DSHEA) of 1994, herbal medicines, which are categorized as dietary supplements, are assumed safe, and do not require authorization from the Food and Drug Administration (FDA) for safety and effectiveness before they are marketed. Nevertheless, a dietary supplement manufacturer or distributor of a supplement with a "new dietary ingredient" may need to go across premarket review for safety and other data. In addition, all domestic and foreign companies that produce package labels or hold dietary supplements must obey the FDA's ongoing Good Manufacturing Practice (GMP) regulations, which outline procedures for ascertaining the quality of supplements planned for sale (FDA 2010, Gao 2010).

In Canada, herbal remedies and traditional medicines for instance, Ayurvedic medicine, must follow the natural health products regulations. The regulations command that a manufacturer, packer, labeller or importer require a preliminary registration with Health Canada prior to starting any such activity. Also, GMPs must be employed to make sure that the product is safe and of good quality. This requires that suitable standards and practices apropos the manufacture, storage, handling, and distribution of natural health products be met (Benzie and Wachtel-Galor 2011, Health Canada 2013).

In Europe, the European Directive 2004/24/EC provides instructions for herbal medicines usage. The directive establishes that herbal medicines released on the market require approval by the national regulatory authorities of each European country. Also, suitable use of these products is due to evidence of safety and efficacy (Calapai 2008).

Standardization of herbal medicines

Traditional herbs and spices are being used as medicines since time immemorial. The widespread use of herbal medicines and issues regarding their safety and efficacy has indisputably increased the need of standardization of these medicines. Guidelines which are set by the WHO are utilized as a standard by most countries. The standardization involves the external (macroscopy/microscopy) and internal examination/ash values, extractive values and many other parameters to identify, verify, and study its chemical composition. Standardization of the therapeutic flora will ascertain indirectly that the flora is protected for their medicinal and nutritive value. Standardization affirms the safety of the therapeutic taxa but effectiveness has to be determined clinically or in the laboratory (Pradhan et al. 2015).

Phytotherapy and its importance

Phytotherapy or herbalism considered as being the world's oldest medical practice used by our ancestors to treat their illnesses is still utilized today by many cultures for medicinal purposes. Herbal medicines involve herbs, herbal materials, herbal preparations, and finished herbal products that contain plants or parts of plants rich in active ingredients which are responsible for disease management (WHO 2008, Laelago et al. 2016).

It is approximated that almost 75% of the medicinal flora used globally were included from traditional medicine. In India, around 70% of current pharmaceuticals are discovered from natural resources and a large number of other man-made analogues has been made from prototype compounds isolated from botanicals (Sen et al. 2011, Pan et al. 2014).

To date, around 80% of cardiovascular drugs are obtained from plant sources. In the year 2005 to 2007, 13 drugs of natural origin were accepted in the United States, and clinical assays on more than 100 natural product-based drugs are ongoing (Pan et al. 2013).

A large number of plants are used in traditional Indian medicine. It was approximated that Ayurveda utilizes 1200 to 1800 plants, Siddha uses 500 to 900 plants, Unani involves 400 to 700 plants, and Amchi utilizes 300 plants (Fig. 10.2). Interestingly, folk healers of India practice more than 7500 therapeutic flora in different medicines (Sen and Chakraborty 2015, Debnath et al. 2015).

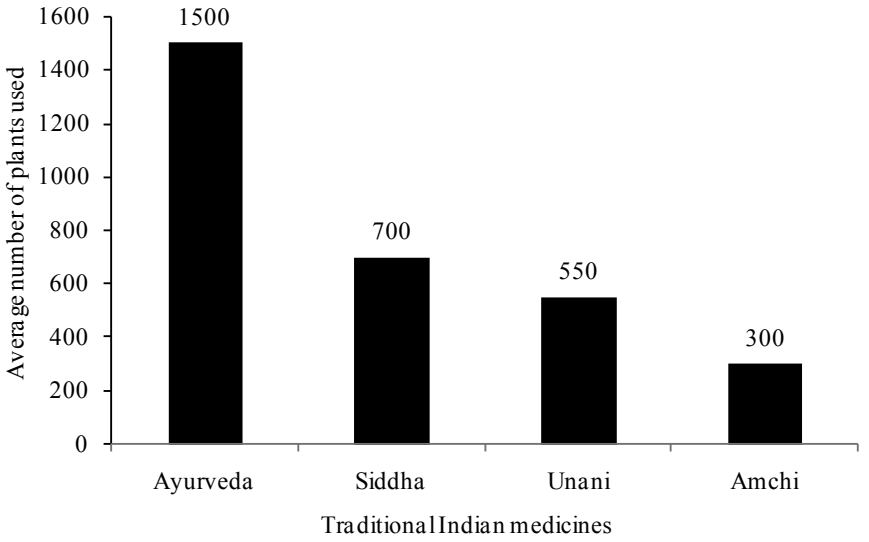


Fig. 10.2 Average number of plants used by Traditional Indian medicines.

Polyherbal formulation and its importance

Polyherbal formulations (PHF) refer to the combination of various medicinal herbs and their active constituents to gain additional therapeutic efficacy, usually known as polyherbalism (Parasuraman et al. 2014). Abana is a polyherbal Ayurvedic formulation containing several herbs such as *Terminalia arjuna, Withania somnifera,* and *Zingiber officinale* to name a few, which protect against hypertensive and coronary heart diseases (Sheela and Shyamala 2000). This positive herb-herb interaction is called synergism whereby poly-herbalism provides some advantages that are absent in single herbal formulation (Parasuraman et al. 2014).

Two types of synergism are known namely pharmacokinetic synergism which occurs when an herb helps in facilitating the absorption, distribution, metabolism, or elimination of other herbs and pharmacodynamic synergism which occurs when active phytochemical constituents having the same medicinal activity act at the same receptor sites. PHF act on multiple targets simultaneously to relief people from symptoms caused by diseases (Chorgade 2007, Parasuraman et al. 2014).

Overall, efficacy, safety, low cost, ubiquity, and better acceptance made PHF a desirable therapeutic alternative which is being used globally (Parasuraman et al. 2014).

Safety issues of traditional medicine

Although TMs are natural remedies and have been used since ancient times, this does not imply that they are always safe. Inappropriate use of TMs or practices can cause serious health problems particularly when used in concomitance with conventional drugs, they may reduce the effectiveness of allopathic drugs and even cause serious drug-herb interactions (Tachjian et al. 2010). Furthermore, intrinsic or extrinsic toxicity of some herbs can produce harmful effects. Ephedra, Aristolochia, and Aconitum herbs are known to cause adverse reactions upon administration (Zhang et al. 2015).

Also, factors such as lack of scientific evidence of safety and effectiveness, quality control, and regulatory surveillance have been identified which complicate the use of TMs (Shi and Klotz 2012, Suroowan and Mahomoodally 2015) and therefore, there is a need to conduct further research in the field of TMs in order to deal with these challenges.

Mechanism of action of some traditional used herbs/spices for CVDs

Welsh onion

Welsh onion (*Allium fistulosum*) is extensively grown in southern China. Fistular onion stalk, derived from welsh onion, leads to a reduction in the average injured area of atherosclerosis and protects the vascular wall and immune cell infiltration. In addition, the crude extract decreases the amount of the inflammatory cytokines interleukin 1 beta (IL-1β), IL-6, Monocyte Chemotactic Protein 1(MCP1), and tumour necrosis factor alpha (TNFα) and suppresses the local activity of the rennin-angiotensin-aldosterone system in the aortic tissue. Also, treatment with the extract represses various local inflammatory signalling pathways by blocking its activation, including phosphorylation of the nuclear factor kappa B (NFκB), Janus kinase/signal transducers and activators of transcription and mitogen-activated protein kinase pathways (He et al. 2014). These facts demonstrate that fistular onion stalk extract may be helpful in reducing atherosclerosis, and the mechanism involves the regulation of the local inflammatory responses.

Chinese ginseng

The root of Chinese ginseng (*Panax notoginseng*), known as Sanqi, Sanchi or Tianqi in East Asian countries, has been identified over 80 variants according to distinct substitute patterns. Five primary saponins namely R1, Rb1, Rg1, Rd, and Re, represent 90% of the total ginseng employed in pharmacological experiments. The protective effect of ginseng in cardiac injury has been demonstrated in numerous studies. It ameliorates rats' heart function evidenced by left ventricular ejection fractions, left ventricular fractional shortening, left ventricular dimensions at end diastole and left ventricular dimensions at end systole (Chen et al. 2011). Furthermore, it diminishes infarction size and blood level of creatine kinase in rats with myocardial ischemia (Yue et al. 2012, Han et al.2013). In addition, it lowers blood levels of lactate dehydrogenase, cardiac troponin I, malondialdehyde, and various cytokines, including TNF-α, IL-1β and C-reactive protein (Han et al. 2013). These studies reveal the curative potentials of ginseng for myocardial infarction.

Hawthorn

The hypolipidemic effect of hawthorn (*Crataegus*) has been explored largely in animal studies. A decrement in blood lipid was observed upon oral administration of a whole hawthorn extract at a dosage of 250 mg/kg/day for 7 days in high-fat diets fed mice (Niu et al. 2011). Then, the specific effects of aqueous and ethanolic hawthorn extracts on lipid profiles were compared. In a high-fat emulsion fed mice, both ethanol and aqueous extracts held anti-hyperlipidemic properties and the ethanol extract displayed more favourable effects than the aqueous extract (Shao et al. 2016). This hypolipidemic effect of hawthorn

mainly contributes to suppression of the development of atherosclerosis which was evidenced by the significantly hindered pathological alterations and decreased intima-media thickness in the arteries (Zhang et al.2013). Thorough investigation indicated that the anti-hyperlipidemic action may be due to the anti-inflammation activities, increase in Peroxisome Proliferator-Activated Receptor alpha (PPARα) to ease β-oxidation-related enzymes in the liver resulting in fat breakdown, greater expression of hepatic LDL receptors leading to a larger entry of blood cholesterol into the liver, and the suppressed cholesterol production and enhanced breakdown of cholesterol to bile acids (Niu et al. 2011, Walden and Tomlinson, 2011, Zhang et al. 2013, Shao et al. 2016).

Saffron

Saffron (*Crocus sativus*) is a stemless plant whose medicinal properties have been sought for more than 4000 years (Srivastava et al. 2010). Saffron's key ingredients include crocin, picrocrocin, safranal, and crocetin (Srivastava et al. 2010, Mehdizadeh et al. 2013) and these compounds give rise to distinct mechanisms of action (Mokhtari-Zaer et al. 2015). The anti-hypertensive benefits of saffron are supported by various reports. According to a clinical study, one week administration of 400 mg of saffron tablets lowered the Systolic Blood Pressure (SBP) and Mean Arterial Blood Pressure (MABP) in healthy participants by 11 and 5 mmHg, respectively (Modaghegh et al. 2008).

Saffron exhibits vasorelaxant activities in distinct animal models. Extracts of saffron petals decreased the BP of male Sprague-Dawley rats in a dose dependent way, most probably by regulating peripheral vascular resistance (Fatehi et al. 2003). Furthermore, saffron stigma extract, and two of its main constituents, crocin and safranal, reduced the effect of MABP in normotensive and desoxycorticosterone acetate (DOCA)-salt induced hypertensive male Wistar rats (Imenshahidi et al. 2010).

Saffron also relaxes non-vascular muscles. Extracts of saffron reduced contractility and cardiac rate of guinea-pig isolated perfused hearts by inhibiting Ca^{2+} channels, opening potassium channels, and antagonizing β-adrenoceptors (Boskabady et al. 2008). Moreover, safranal provides protection in a rat model of myocardial ischemia-reperfusion lesion through enhanced phosphorylation of protein kinase B (Akt)/glycogen synthase kinase-3β (GSK-3β)/eNOS pathway, weakening of the activity of IKK-β/NF-κB, maintaining normal antioxidant reserve and up-regulating the anti-apoptotic pathway (Bharti et al. 2012).

Chinese goldthread

Chinese goldthread (*Coptis chinensis*), is extensively utilized in Chinese folk medicine (Affuso et al. 2010). Through evidence, goldthread, and its prime ingredient, Berberine (BBR), are capable of lowering blood pressure (Affuso et al. 2010, Xiong et al. 2013).

Various mechanisms have been suggested for Chinese goldthread's hypotensive effect. One of which seems to be through enhancement of oxidative stress (Zhang et al. 2011, Wan et al. 2013). BBR is known to scavenge ROS, block NADPH oxidase (Wan et al. 2013), and upregulate the antioxidant enzyme, superoxide dimustase (SOD), in rats with atherosclerotic renovascular disease.

Constituents of goldthread also act by relaxing arterial tissues through endothelial-dependent and independent routes (Affuso et al. 2010). In chronic intermittent hypobaric hypoxic and normoxic animal models, goldthread has been shown to relax norepinephrine-induced contractions in rat isolated thoracic aortic rings (Zhang et al. 2011). The same authors also reported BBR's vasorelaxant activity on KCl-induced contractions using the same models (Zhang et al. 2011).

Apparently, BBR elevates the expression of endothelial Nitric Oxide Synthase (eNOS) with a concomitant rise in NO release that leads to enhanced flow-mediated vasodilation (Affuso et al. 2010, Zhang et al. 2011). This dilation is likely mediated by the vasodilator prostaglandin I_2(PGI_2) as well as the opening of K_{ATP} channels and blockage of Ca^{2+} influx (Zhang et al. 2011). In a clinical study, BBR decreased the formation of endothelial microparticles (EMPs) which are known to induce endothelial dysfunction and pro-coagulant activity in healthy humans (Wang et al. 2009, Affuso et al. 2010). In addition, BBR isolate of Chinese goldthread inhibits endothelial injury (Wang et al. 2009) modulates inflammatory pathways through suppression of transcription factor NF-κB, Vascular Cell Adhesion Molecule 1 (VCAM-1) expression,

Vascular Smooth Muscle Cells (VSMC) proliferation (Affuso et al. 2010, Wan et al. 2013). It also improves lipid profile by reducing total and LDL cholesterol, and cardiac muscle hypertrophy (Zhang et al. 2011).

Green tea

Green tea (*Camellia sinensis*) leaf catechins (GTC) are active polyphenolic compounds that provide vascular protective effects through several mechanisms, namely antioxidative, lipid lowering and anti-inflammatory effects (Velayutham et al. 2008). Environmental pollutants and certain medications may increase the amount of Reactive Oxygen Species (ROS) which lead to vascular damage and development of other circulatory diseases such as atherosclerosis, ischaemic heart disease, hypertension, cardiomyopathies, cardiac hypertrophy and congestive heart failure. In this case, consumption of GTC increases the activity of antioxidant enzymes such as catalase and superoxide dismutase which play key roles in scavenging ROS (Velayutham et al. 2008). Moreover, high blood lipid levels contribute to progression of atherosclerotic plaques and therefore intake of GTC prevents the action of the main enzymes playing a role in the biosynthesis of lipid and also decreases the ability of the intestine to absorb lipid thereby improving blood lipid profile (Velayutham et al. 2008). GTC inhibits vascular inflammation which is involved in the development of atherosclerotic lesions. GTC presents anti-inflammatory activity by preventing adhesion of white blood cells to endothelium and subsequent transmigration via suppression of NF-kB which plays a role in the synthesis of cytokines and adhesion molecules in endothelial and inflammatory cells (Velayutham et al. 2008).

Turmeric

Turmeric (Curc*uma longa*) is an Indian spice with a bright yellow chemical known as curcumin which has cardioprotective effects. Curcumin influences genes leading to cardiac repair and cardiac function following a heart attack. According to a study in the American Journal of Cardiology in 2012, curcumin possibly minimizes post-bypass heart attack risk by 56% (Future Pharm 2017). Turmeric is a great remedy for atherosclerosis because it has the potential to lower blood cholesterol levels and as such minimizing the risk of plaque build-up in the arteries. Turmeric prevents atherosclerosis by the following effects (Singh 2017):

- Curcumin prevents build-up of arterial plaques and arterial blockage by inhibiting LDL cholesterol oxidation in the body.
- Turmeric functions as a vasodilator causing dilation of the blood vessels and therefore reduces the probability of blockage. Moreover, turmeric is capable of preventing aggregation of blood platelets and blocks the formation of blood clots.
- Turmeric possesses anti-inflammatory properties and thereby inhibits inflammatory deposits on the internal walls of the blood vessels.
- Turmeric has antioxidant properties which aid decreased formation of highly reactive free radicals so as to stop further inflammation.
- Turmeric prevents the absorption of cholesterol in the gut thus suppressing LDL cholesterol oxidation in the lining of the blood vessels.

Future prospects of herbal medicine

Despite the growing use and rapid-growing market of medicinal herbs in both developing and industrialized countries, policy-makers, health professionals, and the public are highly concerned about the safety, efficacy, quality, availability, conservation, and any development issues of these herbal products (Ghani 2013).

The public demands for authentication on the safety, efficacy, and quality of herbal medicines. Therefore, to minimize these concerns and to meet the demands of the public, thorough studies on medicinal herbs need to be carried out not solely for their considerable healthcare value but also for the commercial advantages. Interestingly, quite in-depth phytochemical and pharmacological investigations on medicinal herbs are being done worldwide and efforts are ongoing to separate and identify their active chemical components and to substantiate the claims of their efficacy and safety. It has been demonstrated that herbal

medicines are not fully without scientific basis as majority hold the proper chemical constituents and exert the claimed activity (Ghani 2013).

There is strong scientific evidence from randomized clinical studies for use of several medicinal herbs. In spite of the concerns and the demands of the public, there is still an increase in the trend of using herbal therapies and is likely to rise furthermore in the future with more and more scientific evidence of their quality, efficacy and safety coming from the investigators (Ghani 2013).

However, the production, sale, and use of herbal medicines should be officially and legally modulated to ensure quality and safety by established rules and regulations. In most countries, regulations and registration of therapeutic herbs are less developed and their quality is not assured. Therefore, they should be brought under legal control in all countries where they are employed for curative purposes and attempts should be made to make the public aware of the gains and dangers of herbal medicines usage.

Appropriate use of herbal medicine of 'guaranteed quality' will definitely lead to beneficial therapeutic effects on the consumers and lower the risks associated with them. Moreover, use of adulterated herbal ingredients and improper formulation must come to a standstill as they may cause production of poor-quality and dangerous herbal medicines. Therefore, rules and regulations of GMP should be firmly followed in herbal medicines production. With regard to the above data, it may be safely deducted that herbal medicines possess good upcoming prospects and hopefully one day they may come out as good substitutes or better alternatives for man-made conventional medicines (Ghani 2013).

Conclusion

Traditional medicine use among cardiovascular patients is prevalent, with medicinal herbs and spices being the most frequently used remedies. Nonetheless, despite the success of conventional medicines in appeasing the suffering of cardiovascular patients further to improving the quality of life of sufferers, they are not devoid of unwanted side-effects. Hence, interest in TMs has been rejuvenated which are presumed to be safer than allopathic drugs. Even though herbal medicines are useful, many of them lack careful scientific evaluation and this dearth of awareness may eventually lead to toxicity, herb-drug interactions, side effects, and other issues. Therefore, it is recommended that all stakeholders join hands together in order to improve the overall research quality of herbal medicines. As a conclusive note, this chapter provides thorough information on the techniques by which medicinal plants act to prevent CVDs. It is anticipated that these facts will open novel avenues to discover useful new drugs to allay sufferings.

References

Affuso, F., Mercurio, V., Fazio, V. and Fazio, S. 2010. Cardiovascular and metabolic effects of Berberine. World J. Cardiol. 2: 71–77.

Ahmed, K., Jahan, N., Aslam, M., Kausar, H., Khalid, M. and Ali, H. 2014. Dalak (Massage) in unani medicine: a review. IJAYUSH 3: 162–174.

Bharti, S., Golechha, M., Kumari, S., Siddiqui, K.M. and Arya, D.S. 2012. Akt/GSK-3β/eNOS phosphorylation arbitrates safranal-induced myocardial protection against ischemia–reperfusion injury in rats. Eur. J. Nutr. 51: 719–727.

Boskabady, M.H., Shafei, M.N., Shakiba, A. and Sefidi, H.S. 2008. Effect of aqueous-ethanol extract from *Crocus sativus* (saffron) on guinea-pig isolated heart. Phytother. Res. 22: 330–334.

Buttar, H.S., Li, T. and Ravi, N. 2005. Prevention of cardiovascular diseases: Role of exercise, dietary interventions, obesity and smoking cessation. Exp. Clin. cardiol. 10: 229–249.

Calapai, G. 2008. European legislation on herbal medicines: a look into the future. [ONLINE] Available at: https://www.ncbi.nlm.nih.gov/pubmed/18422385. [Accessed 13 August 2017].

Chen, S., J. Liu, X. Liu, Y. Fu, M. Zhang and Q. Lin. 2011. *Panax notoginseng* saponins inhibit ischemia-induced apoptosis by activating PI3K/Akt pathway in cardiomyocytes. J. Ethnopharmacol. 137: 263–270.

Chobanian, A.V., Bakris, G.L. and Black, H.R. 2003. The seventh report of the joint national committee on prevention, detection, evaluation and treatment of high blood pressure: the JNC 7 report. JAMA 289: 2560–2572.

Dupont, S.A., E.F. Wijdicks, G. Lanzino, and A.A. Rabinstein. 2010. Aneurysmal subarachnoid hemorrhage: An overview for the practicing neurologist. Semin. Neurol. 30: 545–554.

Ehrlich, S.D. 2011. Herbal medicine. [ONLINE] Available at: http://www.umm.edu/health/medical/altmed/treatment/herbal-medicine. [Accessed 13 August 2017].

Eleftheriou, D. 2012. Endothelial injury and repair in childhood arterial ischaemic stroke. [ONLINE] Available at: http://discovery.ucl.ac.uk/1378547/1/THESIS%20nov%20pdf.pdf. [Accessed 28 March 2017].

Fatehi, M., Rashidabady, T. and Fatehi-Hassanabad, Z. 2003. Effects of *Crocus sativus* petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. J. Ethnopharmacol. 84: 199–203.

Future Pharm. 2017. Potential Heart Healthy Benefits of Turmeric. [ONLINE] Available at: https://myfuturepharm.com/heart-healthy-benefits-turmeric/. [Accessed 22 October 2017].

Ghani, A. 2013. Herbal medicines: Present status, future prospects. [ONLINE] Available at: http://www.pharmabiz.com/NewsDetails.aspx?aid=78355&sid=21. [Accessed 13 August 2017].

Gurib-Fakim, A. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol. Aspects Med. 27: 1–93.

Han, S.Y., Li, X., Ma, X., Zhang, K., Ma, Z.Z., Jiang, Y. and Tu, P.F. 2013. Evaluation of the anti-myocardial ischemia effect of individual and combined extracts of *Panax notoginseng* and *Carthamus tinctorius* in rats. J. Ethnopharmacol. 145: 722–727.

He, B., Hao, J., Sheng, W., Xiang, Y., Zhang, J., Tian, J., Zhu, X., Feng, Y. and Xia, H. 2014. Fistular onion stalk extract exhibits anti-atherosclerotic effects in rats. Exp. Ther. Med. 8: 785–792.

Imenshahidi, M., Hosseinzadeh, H. and Javadpour, Y. 2010. Hypotensive effect of aqueous saffron extract (*Crocus sativus* L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats. Phytother. Res. 24: 990–994.

Khan, M. and Mensah, G.A. 2010. Epidemiology of Cardiovascular Disease. 2nd ed. Washington: National Academies Press.

Laelago, T., Yohannes, T. and Lemango, F. 2016. Prevalence of herbal medicine use and associated factors among pregnant women attending antenatal care at public health facilities in Hossana Town, Southern Ethiopia: facility based cross sectional study. Arch. Pub. Health 74: 1–8.

Mahomoodally, M.F. 2013. Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. Evid.-Based Compl. Alt. 2013: 1–14.

Mehdizadeh, R., Parizadeh, M.R., Khooei, A.R., Mehri, S. and Hosseinzadeh, H. 2013. Cardioprotective effect of saffron extract and safranal in isoproterenol-induced myocardial infarction in Wistar Rats. Iran J. Basic Med. Sci. 16: 56–63.

Modaghegh, M.H., Shahabian, M., Esmaeili, H.A., Rajbai, O. and Hosseinzadeh, H. 2008. Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. Phytomedicine 15: 1032–1037.

Mokhtari-zaer, A., Khazdair, M.R. and Boskabady, M.H. 2015. Smooth muscle relaxant activity of *Crocus sativus* (saffron) and its constituents: possible mechanisms. Avicenna J. Phytomed. 5: 365–375.

Murray, C.J., Vos, T. and Lozano, R. 2012. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 380: 2197–2223.

Nirosha, K., Divya, M., Vamsi, S. and Sadiq, M. 2014. A review on hyperlipidemia. Int. J. Nov. Trends Pharm. Sci. 4: 1–12.

Niu, C., Chen, C., Chen, L., Cheng, K., Yeh, C. and Cheng, J. 2011. Decrease of blood lipids induced by Shan-Zha (Fruit of *Crataegus pinnatifida*) is mainly related to an increase of PPARα in liver of mice fed high-fat diet. Horm. Metab. Res. 43: 625–630.

Oreagba, I.A., Oshikoya, K.A. and Amachree, M. 2011. Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complem. Altern. M. 11: 1–8.

Parasuraman, S., Thing, G.S. and Dhanaraj, S.A. 2014. Polyherbal formulation: Concept of ayurveda. Pharmacogn. Rev. 8: 73–80.

Pradhan, N., Gavali, J. and Waghmare, N. 2015. WHO (World Health Organization) guidelines for standardization of herbal drugs. IAMJ 3: 1–7.

Rahman, S.Z., Khan, R.A. and Latif, A. 2008. Importance of pharmacovigilance in unani system of medicine. Indian J. Pharmacol. 40: 1–3.

Ravishankar, B. and Shukla, V.J. 2007. Indian systems of medicine: a brief profile. Afr. J. Tradit. Complem. 4: 319–337.

Roger, V.L., Go, A.S., Lloyd-Jones, D.M. et al. 2011. Heart Disease and Stroke Statistics—2011 Update. Circulation. 123: 18–209.

Sharma, S. 2015. Current status of herbal product: Regulatory overview. J. Pharm. Bioallied. Sci. 7: 293–296.

Shao, F., Gu, L., Chen, H., Liu, R., Huang, H. and Ren, G. 2016. Comparison of hypolipidemic and antioxidant effects of aqueous and ethanol extracts of *Crataegus pinnatifida* fruit in high-fat emulsion-induced hyperlipidemia rats. Pharmacogn. Mag. 12: 64–69.

Singh, A. 2017. Turmeric Prevents Plaque Formation Improving Circulation in Atherosclerosis [ONLINE] Available at: https://snec30.com/atherosclerosis-turmeric/. [Accessed 22 October 2017].

Srivastava, R., Ahmed, H., Dixit, R.K. and Saraf, S.A. 2010. *Crocus sativus* L.: a comprehensive review. Pharmacogn. Rev. 4: 200–208.

Suroowan, S. and Mahomoodally, F. 2015. Common phyto-remedies used against cardiovascular diseases and their potential to induce adverse events in cardiovascular patients. Phytomedicine 1: 1–13.

Tabassum, N. and Ahmad, F. 2011. Role of natural herbs in the treatment of hypertension. Pharmacogn. Rev. 5: 30–40.

Tachjian, A., Maria, V. and Jahangir, A. 2010. Use of herbal products and potential interactions in patients with cardiovascular Diseases. J. Am. Coll. Cardiol. 55: 515–525.

Thygesen, K., Alpert, J.S. and White, H.D. 2007. Universal definition of myocardial infarction. Circulation. 116: 1–22.

Torbey, M.T and Bhardwaj, A. 2004. Critical care neurology and neurosurgery. 2nd ed. Totowa: Humana Press.

Velayutham, P., Babu, A. and Liu, D. 2008. Green tea catechins and cardiovascular health: an update. Curr. Med. Chem. 15: 1840–1850.

Wachtel-Galor, S. and Benzie, I.F.F. 2011. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton: CRC Press.

Wan, X., Chen, X., Liu, L., Zhao, Y., Huang, W.J., Zhang, Q., Miao, G.G., Chen, W., Xie, H.G. and Cao, C.C. 2013. Berberineameliorates chronic kidney injury caused by atherosclerotic renovascular disease through the suppression of NFκB signaling pathway in rats. PLoS One 8: 1–8.

Wang, Y., Huang, Y., Lam, K.S., Li, Y., Wong, W.T., Ye, H., Lau, C.W., Vanhoutte, P.M. and Xu, A. 2009. Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilatation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase. Cardiovasc. Res. 82: 484–492.

World Health Organization. 2003. Traditional medicine. [ONLINE] Available at: http://apps.who.int/gb/archive/pdf_files/WHA56/ea5618.pdf. [Accessed 19 December 2017].

World Health Organization. 2003. Traditional medicine. [ONLINE] Available at: http://www.worldhealthorganisation.com. [Accessed 28 March 2017].

World Health Organization. 2005. National policy on traditional medicine and regulation of herbal medicines. [ONLINE] Available at: http://apps.who.int/iris/bitstream/10665/43229/1/9241593237.pdf. [Accessed 28 March 2017].

World Health Organization. 2013. Cardiovascular disease. [ONLINE] Available at: http://www.who.int/cardiovascular_diseases/en/. [Accessed 21 March 2017].

World Health Organization. 2016. Cardiovascular diseases. [ONLINE] Available at: http://www.who.int/mediacentre/factsheets/fs317/en/. [Accessed 26 March 2017].

World Health Organization. 2017. Cardiovascular disease. [ONLINE] Available at: http://www.who.int/cardiovascular_diseases/about_cvd/en/. [Accessed 28 March 2017].

World Health Organization. 2017. Essential Medicines and Health Products Information Portal. [ONLINE] Available at: http://apps.who.int/medicinedocs/en/d/Js2293e/. [Accessed 22 October 2017].

World Health Organization. 2017. Herbal medicine research and global health: an ethical analysis. [ONLINE] Available at: http://www.who.int/bulletin/volumes/86/8/07-042820/en/. [Accessed 19 December 2017].

Xiong, X., Yang, X., Liu, W., Chu, F., Wang, P. and Wang, J. 2013. Trends in the treatment of hypertension from the perspective of traditional Chinese medicine. Evid.-Based Compl. Alt. 2013: 1–13.

Yue, Q.X., Xie, F.B., Song, X.Y., Wu, W.Y., Jiang, B.H. and Guan, S.H. 2012. Proteomic studies on protective effects of salvianolic acids, notoginsengnosides and combination of salvianolic acids and notoginsengnosides against cardiac ischemic-reperfusion injury. J. Ethnopharmacol. 141: 659–667.

Zhang, J., Cui, M., He, Y., Yu, H. and Guo, D. 2005. Chemical fingerprint and metabolic fingerprint analysis of Danshen injection by HPLC–UV and HPLC–MS methods. J. Pharm. Biomed. Anal. 36: 1029–1035.

Zhang, J., Onakpoya, I.J., Posadzki, P. and Eddouks, M. 2015. The safety of herbal medicine: from prejudice to evidence. Evid.-Based Compl. Alt. 2015: 1–3.

Zhang, P., Song, S.J., Liu, W.L., Li, L.L., Zhao, W.L. and Zhang, Y. 2011. Effects of coptischinensis on vasoconstrictive activity of isolated thoracic aorta of normoxic and chronic intermittent hypobaric hypoxic rats. Evid.-Based Compl. Alt. 27: 1–5.

Zhang, J., Liang, R., Wang, L., Yan, R., Hou, R., Gao, S. and Yang, B. 2013. Effects of an aqueous extract of *Crataegus pinnatifida* Bge. var. major N.E.Br. Fruit on experimental atherosclerosis in rats. J. Ethnopharmacol. 148: 563–569.

11

Use of Ethnomedicinal Plants in Primary Health Care

Nélida Soria Rey

Introduction

The World Health Organization (WHO) considers Natural and Traditional Medicine, which includes the treatment with ethnomedicinal plants, as the most natural, safe and effective medicine. WHO, therefore, supports Member States in promoting the use of Traditional Medicines in Primary Health Care (PHC), on the basis of ensuring the safety and quality of the medicine, while recommending professionals and consumers to use ethnomedicinal plants properly because they could be effective as first-line treatment and prevention for conditions such as colds, diarrhea, stomach pains, mild fevers, minor wounds, metabolic and other diseases (Del Toro Garcia and Trapero Quintana 2007).

In recent years, numerous studies have been carried out to define the species used by different human populations to analyze their possible inclusion in Primary Health Care (PHC). The results show that the uses of plant depend on the groups studied and the habitat where communities develop. In a broad perspective, herbal medicine can and should be considered as the shared knowledge and interaction between cultural resources, natural resources and the preservation of biodiversity. In the Latin American context, the experiences applied from an intercultural perspective have generally been characterized by a disconnect of health care treatments from the rest of the societal problems, such as links with the social and economic structure. Ancestral medicine veers away from the generally accepted definition of a modern health care system (Almeida Vera and Almeida Vera 2014).

Although ethnomedicinal plants are used by over 90% of the population in developing countries, their incorporation into PHC is still relegated due to the barriers of health systems, services and personnel, and it is uncommon to integrate in the same service, traditional and allopathic medicines. Thus, many professionals in allopathic medicine, even in countries with a strong history of ethnomedicinal plant use, such as Latin American countries, express great reservations and often serious disbeliefs about the benefits of Traditional Medicine (WHO 2002–2005). Nonetheless, different ethnomedicinal plants make a significant contribution to the health system of many local communities, as they are frequently used by the majority of the rural population and have contributed to experimental studies in the search for safety and effectiveness (Angulo et al. 2012).

It is clear that curative and preventive treatments based on ethnomedicinal plants are the most popular form of traditional medicine and have prevailed over time through oral transmission (Flores 2008).

Faculty of Applied Sciences, National University of Pilar, Scientific Society of Paraguay.
E-mail: nsoria@aplicadas.edu.py

Therefore, one of the current challenges consists in its incorporation within the PHC, for which constant training for the general consumer market and for health care professionals. This training is necessary to finally fulfill one of the fundamental strategies of PHC: their integration with the community in a process of interculturality.

In this chapter, we analyze the parameters that influence the safe and effective use of medicinal plants in primary care, considering that this medicine is affordable to all.

Natural medicine

For centuries, humans have sought out the flora of their surroundings, not only for food but also medicine for diseases that afflicted them. The plants used were almost always related to their environment, and for the said reason they identified numerous medicinal species they used for the cure of their illnesses.

Many of them continue in the present, as for example "aloe" (*Aloe vera*) or "ajenjo (wormwood)" (*Artemisia absinthium*) to which over time properties are added, the result of studies carried out.

The spread of knowledge related to the pharmacological uses of ethnomedicinal plants is generally transmitted orally. Sometimes there is no scientific evidence that allows its use in a safe and effective way. Environmental changes that have taken place globally, such as increasing population in urban areas, led to the modification of the natural habitat and contributed to the disappearance of species and the use of others with the same common name, but without proof that it has the same effect as that attributed to the other species. Climate change also exerts strong pressure and modifies the floristic composition making species considered medicinal by the population disappear.

In addition borders between countries have disappeared and people move freely from one country to another with their "natural medicines". When they are abroad, people substitute their natural medicine with another one that displays similar medicinal properties, using the same common name, thus expanding the number of species used as medicinal.

All these factors contribute to the fact that the number of medicinal species continues to increase, and, in general, we move away from the proposal of the WHO that states: "to contemplate culturally recognized products and practices as a complement to the provision of local health services and to consider to the public health systems of their Member States that are fundamental in determining and facilitating access to safe practices and products" (WHO 2013), because the species used as medicinal are replaced without evidence of their effectiveness and safety by which we cannot assure that the results of the use of the plant is harmless for human consumption.

So, we can no longer speak of "traditional use of a species" or its "harmless" action, because, as mentioned, plants are marketed by their common names and the species are different from those used previously, producing different responses. In Table 11.1 we mention some species that are replaced by others because they have the same common name.

The number of medicinal species that are marketed as medicinal is increasing and the sites in which these products are acquired. Thus, extending the concept that they are not only medicines but also fulfill functions of food and that therefore can be sold anywhere outside of the traditional field of medicine, which

Table 11.1 Example of species that are replaced by each other.

Generic name	Species with the same common name
"Katuava"	*Anemopaegma arvense* (Vell.) Stellfeld ex Souza (Bignoniaceae) *Psidium cinereum* var. *paraguariensis* (Myrtaceae)
"clover"	*Oxalis* sp. (Oxalidaceae) *Amburana cearensis* (Allemão) A.C. Sm. (Leguminosae)
"Francisco Álvarez"	*Luehea divaricata* Mart. (Tiliaceae) *Banara arguta* Briq. (Flacourtiaceae)
"tiger hand"	*Jungia floribunda* Less. (Asteraceae) *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae)
"Cañafístula"	*Cassia grandis* L. f (Leguminosae) *Cassia fistula* L. (Leguminosae)

are pharmacies, such as herbalists, dieticians, naturist stores and informal fairs or street stalls complicating their legal control and the quality of the product offered to the consumer (Acosta et al. 2017).

Although ethnomedicinal plants are effective in primary care and as a first-line medication in stomach conditions, small wounds, control of metabolic diseases (hypertension, diabetes, others), this occurs only when the species used are those with the secondary metabolites that act on these ailments. For this reason, replacing one species for another makes primary care professionals hesitant to prescribe herbal medicines.

We must not forget that many times the uses attributed to medicinal species have been empirical. Traditionally, the shape of the plant's organ was related with the organ of the human body in which it would exert its action. This scheme has varied and currently the use is related not only to morphology but also to flavors or odors, for example, when plants have bitter tastes they are used to combat the increase of glucose in the blood, starting from the assumption that bitter is opposed to sweet.

All of the above leads us to indicate that the use of ethnomedicinal plants is possible in PHC if the parameters that allow their safe and effective use are respected.

Efficacy and safety

The efficacy and safety of ethnomedicinal plants is based on three fundamental pillars that can guarantee their innocuousness:

1) the taxonomic identity assures us, which species is used as medicinal. It is based on taxonomy and includes micrographic methods to certify the identity of the species used,
2) the habitat from which it comes, that is to say its origin or provenance,
3) the dose used, because people have a false idea that excessive consumption does not produce toxic effects, thinking that the medicine by being natural does not produce negative effects and they can consume the amount they want (Fig. 11.1).

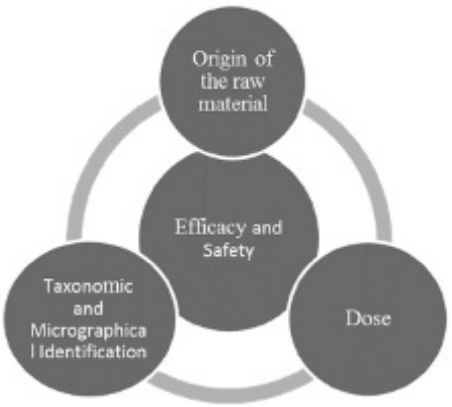


Figure 11.1 Parameters for the efficacy and safety of the use of medical plants.

Taxonomic and micro graphical identification of plants

Ethnomedicinal plants should be marketed by their scientific names ensuring that they are the same species to obtain similar therapeutic responses depending on the medical conditions for which they are used.

The common names with which the species are known may vary from country to country, from region to region, so therapeutic responses may be different, and marketing by the common name may lead to confusion or adulteration, substitutions that can be intentional or not and this constitutes a major problem because it can affect the efficacy of medicinal products and diminish credibility regarding the use of plants as a basic medicine in primary health care (Pocchettino et al. 2008).

Although the most frequently used medicinal organ is the leaf, when the bark, root or rhizome is used, determining the identity of the species becomes complicated and it is common to substitute one

species for another since there are few regulations related to the controls prior to the commercialization of plant organs and when these are sliced or pulverized is even further complicated. For this reason, it is necessary to start working with micrographic patterns to ensure the identity of the species that are used.

That is to say, for the identification of the medicinal species the tools provided by the taxonomy are not sufficient, since in the taxonomy the floral/fruit organs of the species are used for the taxonomic identification; in popular medicine, organs of the species are used that are cut, sliced, pulverized, then one must look for other methods to assure the identity. That is, when you have a part of the plant, the taxonomy can contribute little or nothing to identify the plant species. This is where plant micrograph is of great importance, since it not only contributes the histological parameters that characterize the plant or part of the plant used as medicinal, it also allows the identification and characterization of vegetal powders.

On the other hand, since ethnomedicinal plants are generally harvested from their natural habitat, confusions can occur, with the substitution of one species for another, especially when the collection is done by people without sufficient knowledge and taking into account only the characteristics, which may be similar between two species; that is to say, it could be replaced by scientific ignorance. Also, the substitution can be carried out premeditated, for example the intentional substitution to increase the volume of the species that is commercialized. Consequently, the importance of marketing ethnomedicinal plants should be encouraged by the scientific name, supported by controls that certify the identity of what is sold (Degen et al. 2005).

There are numerous examples of adulteration or falsification of medicinal plants; we mentioned some that can help us understand this problem. The "cangorosa" *Maytenus ilicifolia* (Celastraceae), has thorny leaves and can be confused and replaced by species of *Sorocea bonplandii* (Moraceae), *Jodina rhombifolia* (Santalaceae), also *Tithonia diversifolia* (Asteraceae) and *Jungia floribunda* (Asteraceae) that have similar leaves can confuse one with another. That is to say they are morphologically similar, to differentiate them, it is necessary to carry out taxonomic and micrographic studies that analyze the histological characters that allow the separation of the species (Alonso and Desmarchelier 2007). These species used in folk medicine as a stomach protector, to combat metabolic conditions, although there are few studies related to their effectiveness.

In addition, the use of ethnomedicinal plants from the traditional medicines of India or China, which is completely unknown to western people, is becoming increasingly common, without the corresponding controls that could ensure the efficacy of these products (Veiga Junior et al. 2005), and in general the histological parameters for identification are unknown which makes it difficult to identify species.

According to WHO (2003) reporting of adverse effects related to the use of herbal medicine increased due to the poor quality of them, especially the medicinal plant raw materials and the idea of the general population that the plants are harmless. As mentioned before, people believe there are no adverse effects and they can consume as much and as long as they want. It has been acknowledged, therefore, that not enough attention has been given to guaranteeing and controlling the quality of herbal medicines, making it difficult to use this type of medicine in PHC.

For all of the above, we can affirm that the quality and efficiency of the plants starts with the correct identification of the species by its Latin name. While the standardization of ethnomedicinal plants can be a more complex task, because they contain complex mixtures and the constituents responsible for the effects are often unknown, assuming that they act together.

As mentioned in natural medicine the plant's organ is used, not the entire plant, therefore it is necessary to have physical patterns where the macroscopic characteristics of the medicinal plant materials, such as shape, size, color, surface characteristics, texture, characteristics of the fracture and appearance of the cut surface. However, since these characteristics are judged subjectively and the substitutes or adulterants may resemble the genuine material a lot, it is necessary to corroborate the findings by microscopy and/or physical-chemical analysis, and then the plant histology constitutes the adequate tool for the identification of the drug.

All this leads us to think that the update of the Herbal Pharmacopeia becomes a necessity because our medicine is based on the consumption of ethnomedicinal plants, and in this chapter, we write the characteristics of the organs used as medicine and the identification techniques. Microscopic inspection of medicinal plant materials is indispensable for the identification of powdered materials. The sample

may require studies using chemical reactive and these procedures should be recorded in the monographs of each of the species included in the Pharmacopoeias. It is important to remember that a microscopic examination alone may not always provide complete identification, although when used in association with other analytical methods it can often provide supporting evidence for sample identification (WHO 2005).

Thus, the botanical identity of the plant based drug must be given by the scientific name (genus, species, subspecies or variety, author and family) and the organ considered as medicinal must be described in detail, it may be important in addition to register the common names in the local language, if any.

In addition, specimens of ethnomedicinal plants should be collected and prepared in the form of a herbarium for their preservation, ensuring that they include all the organs required for accurate identification: flowers, fruits, seeds, roots and normal leaves, which should serve as patterns for their correct identification (OMS, UICN, WWF 1993).

This must be supported by the histological descriptions of the medicinal organ and the composition of the powder of the vegetal drug, that is to say we need patterns of the genuine species to base the comparisons and identification.

Origin of medicinal species: natural habitat and cultivation

Ethnomedicinal plants for commercialization are extracted from their natural habitat or cultivated. In almost all countries of Latin America, ethnomedicinal plants come mostly from their natural habitat, and few are grown, probably due to the lack of knowledge of agricultural techniques for production. The species introduced and acclimated, in general, the conditions for their cultivation are known and it is important to remember that the ethnomedicinal plants constitute a significant commercial field, the reason why the extensive exploitation of the species can take them to the limit of the extinction (WHO 2003).

On the other hand, numerous efforts have made in recent years to draw attention to the problems nature faces because of deforestation and the destruction of ecosystems, mainly due to anthropogenic actions. The loss of biodiversity with the consequent disappearance of species depends on factors associated with common economic and social problems in developing countries. The disorderly growth of populations due to urban and rural development without adequate planning processes causes pressures on available natural resources. On the other hand, overexploitation, livestock, industrialization, construction of road infrastructures and other characteristics have contributed significantly to the processes of extinction. In addition, as we know, the problems that affect the conservation of biodiversity contribute to the disappearance of species considered medicinal and in many cases, are replaced by others with similar characteristics.

As already mentioned, in some Latin American countries, the number of plants used for medicinal purposes is increasing, Basualdo et al. (2003, 2004) mentioned that in Paraguay, in their capital and in the metropolitan areas, 266 species are traded for medicinal purposes, used to combat, prevent or cure 57 diseases; Pin et al. (2009) cited 500 medicinal species used for preventive and therapeutic purposes.

These lists include species introduced, acclimatized and native, not to mention the origin that is if they come from crops or are extracted from their natural habitat. In other countries of the Americas, there is a similar situation, in Colombia, according to Duque Villegas (2003), from 100 to 243 medicinal and aromatic species are sold in the marketplaces of the city of Bogotá; of them, between 50 and 60% correspond to native species (it is not known if they are wild or cultivated), while 20 and 40% are naturalized, coming from other places.

One of the most frequent problems when collecting medicinal species from wild populations is the confusion of species and when the "originals" are replaced with other species or parts of other plants due to incorrect identification. Sometimes the substitution may be intended to increase the volume of marketing and with the certainty that the user will not perceive the difference.

Another problem that we mention when we talk about quality is the contamination with agrotoxics because it is close to agricultural production sites on a large scale, where it is sometimes fumigated, even using small airplanes so that the product used reaches all cultivated areas. These circumstances may adversely affect the safety of products and cause health problems in people who consume these herbs. This type of contamination occurs in natural or cultured populations.

The safety and quality of medicinal vegetable raw materials and finished products made from this raw material depends on factors that can be classified as intrinsic (genetic) or extrinsic (species selection, harvesting methods, cultivation, harvesting, post-harvest processing, transportation and storage practices) (Fig. 11.2). Inadvertent contamination by microbial or chemical agents during any stage of production may also compromise the safety and quality (WHO 2003).

As mentioned above, obtaining the raw material from its natural habitat can produce the substitution with native species similar to the one harvested, an example is "yerba mate" *Ilex paraguayensis* (Aquifoliaceae), a species used to prepare mate, cooked mate, a brew that is made with the leaves of the species mentioned, and is used as food in the southern part of Latin America, Paraguay, Brazil, Argentina, Uruguay. Natural populations called "yerbales" when harvested were adulterated with other species in a fraudulent way to increase the amount for commercialization (Keller and Giberti 2011, Horianski et al. 2012).

The cultivation of ethnomedicinal plants for use as raw material offers the advantage that it is possible to know which species is cultivated, that is to say, the taxonomic identity, the genetic traits and the pharmacognostic factors are handled, which determine the best moment for the collection of the raw material, that is to say when it has more quantity and quality of its secondary metabolites.

In addition, by cultivating selected species, extrinsic factors can be managed to obtain abundant raw material, which is homogeneous and of high quality. It is possible to control some of the variables that affect the production (nutrient supply to the soil, pest control, humidity) and, therefore, to improve the yield in active principles. In crops, in general, plants develop in the same way, which facilitates the collection, drying and in some cases the extraction process (WHO 2005).

Knowing the harvesting period is fundamental to obtain good quality raw material, ethnomedicinal plants should be harvested during the optimum season or period to ensure obtainment of the appropriate secondary metabolites of the best possible quality. The harvesting season depends on the part of the plant to be used. In addition, it is known that the concentration of components with biological activity, as well as that of the undesired secondary metabolites, varies according to the stage of growth and development of the plant, for example when harvesting flower, it is good to it collect early in the morning, before they fully open. That is to say, the best time to harvest (the optimum season and times of day) can be managed according to the quality and quantity of the components with biological activity, and not only the total yield in vegetal matter of the parts of the ethnomedicinal plants subject to production.

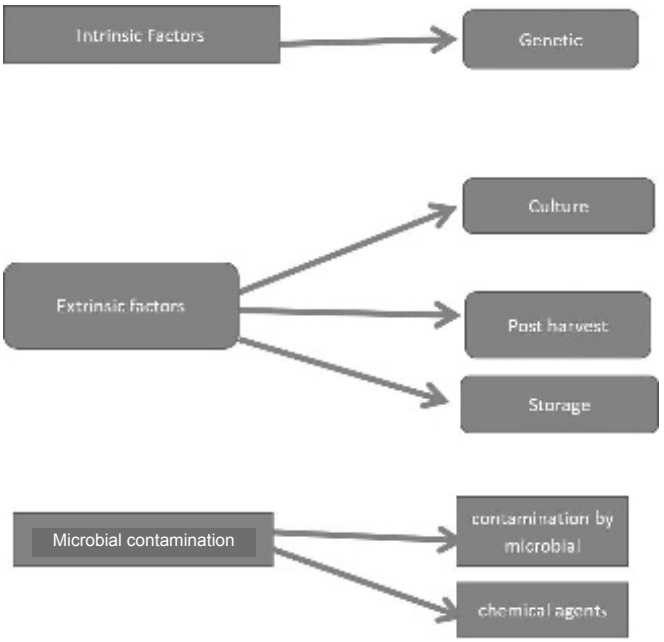


Fig. 11.2 Factors that influence the safety and quality of plant material.

During the harvest, care must be taken to prevent foreign matter, other herbs and toxic plants from mixing with the harvested plant material. Ethnomedicinal plants should be harvested in the best possible conditions, in the absence of dew, rain and exceptionally high humidity levels. If the harvest is carried out in wet conditions, the harvested material must be transported immediately to a drying plant under the roof to accelerate drying and thus avoid the possible detrimental effects of high humidity levels, which encourage microbial fermentation and molding, decreasing the quality of the raw material (WHO 2003).

Microbial contamination

Bacterial or fungal contamination of ethnomedicinal plants represent a serious problem because of the risk involved for the health of the patient and for the economic losses generated when they are raw material for the production of pharmaceuticals, food or cosmetics. This type of contamination is closely linked to the supply chain, where some part of the process fails to obtain products that deviate from the characteristics of what is sought.

Inappropriate values of microbial contamination are often due to a poor drying or storage process, which can lead to both the proliferation of pathogens and the production of toxins of different nature (aflatoxins*, E. coli* enterotoxin) that are capable of causing serious complications in users (Vanaclocha and Cañigueral 2006).

Microbiological control has two very important aspects:

1. Hygienic and Sanitary Quality, that is to say that the raw materials do not contain pathogenic microorganisms for human health.
2. Commercial quality, that the raw material does not present characteristics that make it unsuitable for its use.

As stated, microbiological control is an important part of the quality of ethnomedicinal plants used in primary care.

Conservation of medicinal species

As mentioned, for marketing, ethnomedicinal plants are harvested from their natural habitat. According to Soria and Basualdo (2015), 67% of the plants mentioned as medicinal in Paraguay, come from their natural habitat, while only 33% is cultivated, so the conservation of these plants is an aspect that must be addressed, since extensive and uncontrolled extraction may jeopardize the sustainability of species over time. In addition to the factors that affect the conservation of the species, there is another one, directly linked to the medicinal organ used; so, when the root, rhizome or part of them is used, the plant must be extracted completely even if only a part of these subterranean organs is used.

This contributes to the overexploitation to which they are submitted for commercial use, especially, when they constitute raw material of some herbal medicine, in more industrialized processes for the pharmaceutical, food, cosmetic industry. The development of policies for the conservation of species in general, and of medicinal species in particular, becomes a necessity, for which studies should be carried out to determine the degree of threat of each of the species that could be used in PHC.

The conservation status of native species is a global concern. It is necessary to understand the potential threat of survival for species before entering a cropping program. Therefore, it is recommended that prior to their use in the PHC; a count of raw material quantity is conducted to ensure sustained effectiveness of a cropping program.

Due to the concern for species conservation, the Strategic Plan for Biological Conservation 2010–2020 and Aichi's goal number 12 propose that "by 2020 the extinction of identified endangered species would have been avoided and their conservation status would have been improved and sustained, especially for the most declining species" and indicates the need to identify species at risk of extinction (Soria and Basualdo 2015).

Conservation of ethnomedicinal plants is a major risk due to the lack of knowledge we have about them. In order to effectively use and conserve ethnomedicinal plants, it is essential to know precisely

the information about the species, what the correct scientific name is, the number of individuals in the population, whether the extraction is done from their natural habitat, and the distribution of the population. There is no known accepted global list of ethnomedicinal plants that are used today. Many ethnomedicinal plants are poorly identified. For this and other reasons, any national program on the use and conservation of ethnomedicinal plants should include an herbarium specimen conservation mechanism that allows the identification of ethnomedicinal plants in the country, their distribution and their scarcity or abundance.

Dosage

In general, medicinal herbs are prepared in infusion or decoction for their consumption, the procedure is very important for the extraction of the active substances contained in them. When it comes to soft organs (leaf, flower, and aerial), the preparation is done by means of infusion, that is to say the water is boiled and it is fed on the vegetable, sometimes it is recommended to cover to avoid that the essential oils to be vaporized. When a hard part is used (bark, stem, root, and seed) it is boiled in the water for some minutes that can vary between five or ten, the resulting liquid is filtered and the decoction obtained is drunk. In general, the amount of the plant to be used depends on the plant organ used and the amount of water used is related to the contents of a 150 ml cup (Table 11.2). The extracted product is very little with this procedure (Tyler and Robbers 2003, ANVISA 2011).

With regard to species activity, it is important to remember that the use of plants may interact with allopathic drugs. There are studies showing that the simultaneous consumption of medicine and organs, parts or products coming from ethnomedicinal plants may lead to interactions, which further motivates the need to recognize that plants should be treated as medicine. It is important to know the attitudes of users and health personnel regarding the consumption of ethnomedicinal plants as a medicine (Rodríguez Ramos 2014).

In general, in Latin American countries, with a strong tradition in the use of ethnomedicinal plants, 99% of the population that goes to the health services, admit they use ethnomedicinal plants for medicinal or preventive purposes, although in general they do not inform health personnel of their use because they consider that consumption is harmless. It is important to remember that this medicine constitutes the only form of traditional medicine widely accepted by the population in all countries of Latin America.

One of the current trends in medicine has been to incorporate Traditional Medicine into professional practice, not as an alternative method motivated by economic causes, but as a scientific discipline that must be studied, perfected and developed permanently, for its ethical and scientific advantages, because it constitutes a means of recovering the cultural heritage of the people, which is in danger of disappearing with the advance of "modern medicine" (Torres and Quintana 2004).

All of the above leads us to mention that public policies, which favor multidisciplinary investigations in ethnomedicinal plants, are needed, prior to their use in primary care to ensure the use of a more effective and low-cost medicine.

Table 11.2 Relationship of plant organ/grams/mL of water.

Vegetable drug	Weight in grams	mL
Dry leaves	3–4	150
Inflorescence	6–9	100
Roots, bark	2–5	100
Flowers	1–3	150
Fruit peel	6	150
Aerial part	2–5	150

Examples of effects of joint use of plants and allopathic drugs

In recent years, adverse effects related to the use of plants in conjunction with medicinal allopathic have increased. Let us look at some examples. When antidepressant treatment and concomitant use of medicinal species as "St. John's wort" also known as "wort" (*Hypericum* sp., Clusiaceae), a drug popularly used as an antidepressant, can cause complications such as decreasing the antidepressant effects of the chemical drug and also provoke contrary effects to those sought and induce a strong anxiety in the users. In addition, this herb is photosensitizing in animals and although there are no studies for humans, it is recommended that people not be exposed to sunlight (Tyler and Robbers 2003).

Valerian (*Valeriana officinalis* L., Caprifoliaceae), popularly used as a sleep inducer, sedative, moderate tranquilizer, used in conjunction with barbiturates, benzodiazepines and/or alcohol can cause intestinal bleeding, potentiate the action of barbiturates and reduce symptoms of abstinence from benzodiazepines.

In addition, the use of anti-coagulants can also be affected by the consumption of ethnomedicinal plants, such as ginger (*Zingiber officinale* L., Zingiberaceae), which can cause hemorrhages caused by its consumption, considering that this plant is used in food as spice for its flavor. It can also produce high blood pressure.

The *Ginkgo biloba* L., is a species considered as a living fossil. It is the only living representative of the Ginkgoaceae family of the order of the Ginkgoales; this species coexisted with dinosaurs, in the Jurassic era from where it comes to our days maintaining its medicinal properties, i.e., preventing and fighting the effects of strokes, stimulating circulation, is also considered to be an antioxidant and anti-inflammatory. This species should not be consumed along with antidepressants, anticoagulants, aspirin, warfarin, eparin and it is recommended that epileptics do not use it. The reported side effects are interference with serotonin, production of greater bleeding by potentiating the effect of anticoagulants, stomach discomfort, and migraine.

Also, the "confrei" (*Symphytum officinale* L. Boraginaceae), a species that is used in traditional medicine for healing effect, possesses pyrrolizidinic alkaloids which are hepatotoxic and can produce carcinogenic cells. After several cases of death caused by cirrhosis resulting from veno-occlusive liver disease triggered by these alkaloids, the use of this species was banned by the WHO plant safety agencies.

There are other species that produce undesirable effects due to its secondary metabolites, such as *Senecio grisebacchii* Baker (Asteraceae) that is used in the traditional medicine to "renew the blood", coinciding with beginning of the month of August, end of the winter, in the southern hemisphere, this species possesses pyrrolizidine alkaloids that cause hepatic damages. Likewise, "jurubeba", *Solanum paniculatum* L. (Solanaceae), can cause irritation of the gastrointestinal mucosa. Likewise, *Aristolochia* species are attributed with diuretic and anti-infective properties in the urinary tract, antirheumatic, sedative and sometimes is part of slimming preparations. Among its secondary metabolites, it has the aristolochic acid, consumption of which can cause renal problems (Arango Toro 2005).

"Salvia", *Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson (Verbenaceae), has shown good results for its use as an antispasmodic, hypotensive and high dose has not reported toxic effects or intolerance. It has experimentally demonstrated analgesic, hypotensive, smooth muscle relaxant, and anti-fungal activities against *Candida albicans* .This last action validates the uses in cases of infections where this microorganism is involved. The other tested effects act in a beneficial way in the baths given to women during the puerperium. The combination of *Lippia alba* extract and paracetamol in rabbits increases the toxic effect of paracetamol on the liver, so it should not be administered in conjunction with this drug (Argueta and Cano 1994, Gonzalez and Naranjo 2000).

Care group: Pregnant women, infants, children, and the elderly persons

In relation to the side effects that ethnomedicinal plants may have, pregnant women, infants and children under two years should avoid the consumption of ethnomedicinal plants, especially when there is no safety

study. Nursing mothers run the risk that the drug may pass even though it is in small proportion to the child through milk, and because the organs of the babies are not yet fully strengthened, can cause adverse effects.

There is also another important group of users: the elderly. Due to their age kidney functions decreases, difficulty in the absorption, distribution and elimination of secondary metabolites of the plants, which can lead to poisoning, frequently confused because people assume that products of plant origin are "harmless".

Ethnomedicinal plants in Primary Health Care

Everything mentioned, leads us to understand that when we talk about ethnomedicinal plants in primary care, we are talking about medicines and therefore we must consider and manage them as such respecting the parameters that allow us to use them in first line treatment and prevention, especially in the initial phases of colds, diarrhea, stomach pains, epidermal problems, mild fevers, metabolic diseases (hypertension, type II diabetes and other metabolic diseases), and is also a form of affordable, accessible and available care (Soria and Ramos 2015).

So, which plants meet the requirements for use in PHC?

Many drugs come from traditional medicine and have been used for centuries, providing some assurance of their safety, mainly when acute toxicity is concerned. Probably, that ancestral use has contributed to rooting in the population the generalized perception that natural is synonymous of harmless. However, although plant drugs and derivatives often have a broad therapeutic range, they are not exempt from possible adverse effects, interactions and contraindications. Hence, the evaluation of their safety should be carried out with the criteria applied to other medicinal products and should be supported, whenever possible, in the existence of relevant scientific documentation on their toxicity, side effects, interactions, and contraindications. Many species have passed the test of efficacy and safety and could be used as a base drug; it is convenient to remember that these species have a recommended dose and that their indiscriminate use can cause adverse effects.

Let us look at some examples of ethnomedicinal plants that could be used in primary care, as long as the parameters for safety and efficacy to which we referred in this chapter are respected:

"Aloe" *Aloe saponaria* Haw. (Asphodelaceae), is a species used since antiquity. It is mentioned in the Bible, is native to North Africa and introduced in America, where it is grown in abundance. In external use, it can help healing wounds, for which the mucilage of the fresh leaf on the wound is allowed to drain. It can also be used to treat zits, acne, eczema, ulcers, itching of the skin, treatment to be repeated 3–4 times a day. The adverse effect reported was photosensitivity and contact dermatitis (Alonso 2004).

"Boldo, false boldo" *Plectranthus barbatus* Andrews (Lamiaceae), the aromatic leaves of this species are used as digestive medicine. It is recommended not to administer to people with hypertension, carriers of obstruction of the bile ducts, patients who use drugs for the central nervous system; can lower blood pressure and cause gastric irritation. Consumed with allopathic products such as metronidazole or disulfiram can decrease the effects of these drugs. It should not be used by pregnant women, infants and children under 2 years of age (Anvisa 2011).

"Boldo" *Peumus boldus* Molina (Monimiaceae). This species is used in hepatic, vesicular ailments and it is used for its digestive properties. At the usual doses 2–3 g of dry leaf in 150 mL of water, boldo infusion is well tolerated. However, because of its choleretic action, it should not be used in cases of obstruction of the bile ducts or in severe liver diseases. Some of the active ingredients have oxytocin activity and should not be given to pregnant women. Neither should be administered to children younger than 2 years (Alonso 2004).

"Cangorosa" *Maytenus ilicifolia* Mart. ex Reissek (Celastraceae), the blending of the leaves and the root bark is used to combat dyspepsia, heartburn, gastritis. It is prepared in infusion of 3–5 g of dry drug in 150 mL of water or 5–7 g of root bark in 200 mL water and it is drunk right after main meals. Pregnant women, infants and children under 2 years should not consume it. Prolonged use can cause nausea and/or a strange taste in the mouth (Sharapin et al. 2006).

"Guayaba" *Psidium guajava* L. (Myrtaceae), the leaves are used to treat pharyngitis and in external use to wash wounds, for which the decoction of 50 g of fresh leaves in 1 L of water is prepared and the wounds are washed 3 times per day. To treat pharyngitis the person must gargle up to 3 times a day, without swallowing. Use for short periods, no more than 30 days. Do not administer to pregnant women, infants and children under 2 years of age (WHO 2010).

"Kava kava" *Piper methysticum* G. Forst. (Piperaceae), is used in alternative medicine in the treatment of anxiety, insomnia, depression, attention deficit disorder, and has shown positive results when used in the prevention of withdrawal symptoms sedatives from medicines like Valium, Xanax and others. Continued use of this species can affect the liver and lead to hepatitis, cirrhosis and liver failure. The use of this species is considered unsafe, especially because of the abuse of it. People do not limit their use to the recommended dose (Alonso 2004).

"Guaco" *Mikania glomerata* Spreng. (Asteraceae), the leaves are used as an expectorant, according to the form published by ANVISA of Brasil (2011), boil 150 mL of water, pour on 3 g of dry leaf, and drink twice day. Do not use with non-steroidal anti-inflammatory drugs, as their use may interfere with blood clotting. Higher than recommended doses can cause vomiting and diarrhea.

The leaf of sour orange *Citrus auriantium* L. (Rutaceae) is used in traditional medicine as antispasmodic and sedative. In external use to combat foot odors, 10–15 g of fresh leaves are squeezed, macerated in cold water and drank during the day. The decoction of 50 g/l of leaves boiled in water for 10 minutes is used to wash the feet daily, decreasing the odor. It is not recommended to administrate it to pregnant women, infants and children under 2 years (ANVISA 2011).

"Fennel, hinojo" *Foeniculum vulgare* L. (Apiaceae), is used as digestive, carminative and diuretic. It is prepared in infusion, 2–3 g of seed in 150–200 mL of water and 2 cups a day is drunk after main meals. Recommended doses do not produce adverse effects. High doses have demonstrated to have the emenagogo effect, for which reason it should not be administered during pregnancy and the lactation period. The essential oil can cause, in doses greater than 1 ml, convulsive or hallucinogenic effects. Do not administer to children under 2 years of age. Do not consume for prolonged periods, that is to say for more than three months on an ongoing basis. The aqueous extract can produce photosensitivity, so sunbathing is not recommended when drinking the infusion of this species.

"Breaking stone" *Phyllanthus niruri* L. (Euphorbiaceae), the aerial part of the plant is used as a diuretic and to combat kidney stones. An infusion of 3–4 g is prepared in 150 mL and is drunk up to 3 cups a day. It is not recommended to use it in prolonged treatments since it can produce diarrhea, hypotension and marked diuresis. It is not advisable to administer it to pregnant women, infants and children under 2 years of age (ANVISA 2011).

The species *Scoparia dulcis* L. (Plantaginaceae) is used in popular medicine as digestive medicine; studies show that the species has antispasmodic effect in stomach cramps. There are still few studies regarding the toxic effects, but it is believed that it could potentiate the effect of barbiturates and act as a selective inhibitor of serotonin reuptake. There are numerous studies related to the use of this species for its antidiabetic action for which it has shown promising effects (De Farias Freire et al. 1993, Pari and Latha 2004).

It is not intended to make an extensive listing; only some species are shown that could be useful in primary care mentioning its properties and the care that should be taken for their use.

It is important to remember that the effect of ethnomedicinal plants is not immediate, so it cannot be used in acute conditions. In addition, it is recommended that consumption of the same species does not last for more than six months, which should be followed by a six-month waiting period as well. This is because the metabolites are transformed into the liver and are generally removed by the urinary tract. This waiting period allows the body to recover from the stress that could have caused the prolonged use.

An important aspect that we must not forget is that vegetable drugs can cause side effects that are manifested with symptoms like nausea, stomach pain, diarrhea, headache, when any of these symptoms are felt, the use of it should immediately stop. If symptoms persist, contact a physician, who should be informed of the species used and the time of consumption to find the appropriate solution.

Conclusion

Ethnomedicinal plants can be used in primary health care as a more attainable, affordable, safe and effective medicine if the conditions for its use are maintained.

Efficacy and safety are two main aspects to take into account when using ethnomedicinal plants and they depend on: (i) the taxonomic identity (ii) the habitat from which it comes (iii) the dosage used.

The extraction of the species from their natural habitat can cause confusion between species of similar morphological characteristics, resulting in adulteration and consequently different therapeutic responses. Vegetable matter from crops has the advantage that the pharmacognostic factors can be handled and it is also possible to obtain raw material of good quality, suitable for medicinal use.

It is necessary to continue the studies analyzing all of the above-mentioned factors. Researches should be collaborating together through an intercultural approach that allows them to analyze and ascribe solutions to community health problems in a dynamic viewpoint which, links existing socioeconomic structures and ancestral medicine to the official health system that allows safe and effective use of ethnomedicinal plants.

References

Acosta, M.E., Ladio, A.H. and Vignale, N. 2017. Medicinal plants traded in San Salvador de Jujuy city (Argentina) and its botanical quality. Bol. Latinoam. Caribe Plant. Med. Aromat. 16(1): 34–52.

Almeida Vera, L. and Almeida Vera, L. 2014. Rationale of the Ecuadorian Intercultural Management Model in Primary Health Care. Medisan 18(8): 1201–1214.

Alonso, J. 2004. Treaty on Phytopharmaceuticals and Nutraceuticals. Ediciones Corpus. Rosario Argentina, pp. 131.

Alonso, J. and Desmarchelier, C. 2007. *Maytenus ilicifolia* Martius (Congorosa). Bol. Latinoam. Caribe Plant. Med. Aromat. 6(1): 11–22.

Angulo, F.A., Rosero, R.A. and González Insuasti, M.S. 2012. Ethnobotanical study of medicinal plants used by the inhabitants of the village of Genoy, Municipality of Pasto, Colombia. Rev. Univ. Salud 14(2): 168–185.

ANVISA. 2011. National Formulary of Phytotherapy of Brazil. Brasilia, 560 p.

Arango Toro, O. 2005. The use of medicinal herbs can produce serious nephrological and urological problems. Actas Urol. Esp. 29(8): 801–802.

Argueta, V.A. and Cano, J.A. 1994. Atlas of traditional Mexican medicine plants. México: Instituto Nacional Indigenista.

Basualdo, I., Soria, N., Ortíz, M. and Degen, R. 2003. Medicinal use of plants commercialized in the markets of asuncion and greater asunción. Rev. Soc. Cient. Parag. 14: 5–22.

Basualdo, I., Soria, N., Ortíz, M. and Degen, R. 2004. Medicinal plants marketed in the markets of asuncion and greater asunción. Rojasiana 6(1): 95–114.

Basualdo, I. and Soria, N. 2015. Medicinal plants marketed in the municipal market of the city of Pilar, Dpto. Ñeembucu, Paraguay. Dominguezia 30(2): 47–53.

De Farias Freire, S.M., Da Silva Emim, J.A., Lapa, A.J., Souccar, C. and Brandao, L.M. 1993. Analgesic and anti-inflammatory properties of *Scoparia dulcis* L. extracts and glutinol in rodents. Phytother. Res. 7(6): 408–414.

Del Toro García, G. and Trapero Quintana, Y.M. 2007. Natural medicine and health system in Cuba. Bol. Latinoam. Caribe Plant. Med. Aromat. 6(3): 48–51.

Degen, R., Soria, N., Ortiz, M. and Basualdo, I. 2005. Problems of common names of medicinal plants marketed in Paraguay. Dominguezia 21(1): 11–16.

Duque Villegas, A. 2003. National survey of medicinal and aromatic plants an approach to the market of pmya in Colombia 26 p.

Flores, A. 2008. Conflicts in the use of Traditional Medicine. Reg. Soc. XX(43): 213–217.

Gonzáles, A. and Naranjo, S. 2000. Possible hepatotoxicity induced by the association of *Lippia alba and* acetominofen in rabbits. Conaplamed Info. 16: 25–35.

Horianski, M.A., Castrillo, M.L., Tayagui, A.B. and Jerke, G. 2012. Microbiological quality of canchada yerba mate. Rev. Cienc. Tecnol. 17: 30–33.

Keller, H.A. and Giberti, G. 2011. First record for the Argentine flora of *Ilex affinis* (Aquifoliaceae), substitute of the "yerba mate". Bol. Soc. Argent. Bot. 46: 1–2.

OMS, UICN, W.W.F. 1993. Guidelines on Conservation of Medicinal Plants, 34 p.

Pari, L. and Latha, M. 2004. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. BMC Complem. Altern. M. 4: 16.

Pin, A., González, G., Marín, G., Céspedes, G., Cretton, S., Christen, P. and Roguet, D. 2009. Medicinal Plants of the Botanical Garden of Asunción. Asunción, Paraguay, 441 pp.

Pochettino M.L., Arenas, P., Sánchez, D. and Correa, R. 2008. Traditional botanical knowledge, commercial circulation and consumption of medicinal plants in an urban area of Argentina. Bol. Latinoam. Caribe Plant. Med. Aromat. 7(3): 141–148.

Rodríguez Ramos, R. 2014. Medicina Naturista y Atención Primaria de Salud. Experiencia Cubana. Available in: http/: www.uva.org. Ar/cuba.

Sharapin, N., Alonso, J.R., Henriquez, A., Zuanazzi, J.A., Mentz, L.A., Nunez, E., Apel, M. and Cañigueral, S. 2006. Cangorosa Leaf (Maytenus ilicifolium). Rev. Fitoterapia 6(1): 29–43.

Soria, N. and Basualdo, I. 2015. Conservation of medicinal species in Paraguay (Part I). Dominguezia 31(1): 41–47.

Soria, N. and Ramos, P. 2015. Use of medicinal plants in primary health care in Paraguay: some considerations for safe and effective use. Mem. Inst. Investig. Cienc. Salud 13(2): 8–17.

Torres, I.B. and Quintana, I.J. 2004. Comparative analysis on the use of medicinal plants in traditional medicine in Cuba and Canary Islands. Rev. Cubana Plant. Med. 9(1): 9–14.

Tyler, V.E. and Robbers, J.E. 2003. Las hierbas medicinales de TYLER. Uso terapéutico de las fitomedicinas. Editorial Acribia SA, 254 p.

Vanaclocha, B. and Cañigueral, S. 2006. Possible hepatotoxicity of rhizome of cimicífuga. Rev. Fitoterapia 6(2): 129–135.

Veiga Junior, V.F., PintoI, A.C. and Maciel, M.A. 2005. Medicinal plants: safe cure? Quim. Nova 28(3): 1–15.

World Health Organization (WHO). WHO strategy on traditional medicine 2002–2005. Geneve, Suiza. 1–75 pp.

World Health Organization (WHO). 2003. WHO Guidelines on Good Agricultural Practices and Collection (BPAR) for Medicinal Plants. Geneve, Suiza, 79 p.

World Health Organization (WHO). 2005. Quality Control Methods for Medicinal Plant Materials. Geneve, 122 p.

World Health Organization (WHO). 2010. Who Monographs on Selected Medicinal Plants 4. pp. 108–117.

World Health Organization (WHO). 2013. WHO Strategy on Traditional Medicine 2014–2023. Geneve, Suiza, 75 pp.

12

Secondary Metabolites of Some Ethno-medicinal Plants of Arunachal Pradesh, India

Pallabi Kalita Hui,[1,*] *Debmalya Das Gupta*[1] and *Hui Tag*[2]

Introduction

The North Eastern (NE) region of India represented by seven Indian states, viz. Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland and Tripura is a 'Global Biodiversity Hotspot' and represents one of the highest biodiversity of the Indian subcontinent. The region is often referred to as the *Seven Sisters* and Sikkim has also now been included in North eastern states. It is ecologically represented by the Eastern Himalayan biome and is rich in a number of endemic flora and fauna endowed with a rich biodiversity. Arunachal Pradesh popularly called as "*Land of Rising Sun*", falls under a geographical coordinates of 26°28' to 29°30' North Latitude and 90°30' to 97°30' East Latitude spreading over a geographical area of 83,743 sq. km. It shares borders with Assam and Nagaland in the South, Tibet (China) in the North and North East, Bhutan in the West and Myanmar in the East. The state being primarily a hilly tract is nestled in the foothills of Himalayas situated on the Great Eastern Himalayan Mountain Range and is recognized as the 12th Global Biodiversity Hotspots. It covers a vast diversity of flora and fauna making it one of the richest biotic province of the Republic of India. Parallel with this, nature has exceedingly endowed the state with rich bio-resources along with the second largest area under forest cover, i.e., the total area of 68,045 sq.km in the whole of India after Madhya Pradesh (Anonymous 2008).

Arunachal Pradesh is a tribal state comprises of 19 districts inhabited by 26 major tribes and 110 sub-tribes of diverse culture and lifestyle, with a traditionally rich Indigenous Knowledge System (IKS), across different geographical regions since time-immemorial (Fig. 12.1) (Tag et al. 2005). The majority of the local tribal communities originally belonged to Mongoloid racial stock but their long history of migration coupled with geographical isolation has altogether contributed to varied distinctive characteristics which are reflected in their language, dress and customs and customary laws amongst different tribes and sub-tribes. The richness of life form, i.e., the flora and fauna that occur in the pristine forest presents a panorama of

[1] Department of Biotechnology, National Institute of Technology Arunachal Pradesh (NIT AP), Yupia-791112, Papum Pare, Arunachal Pradesh, India.
[2] Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh-791112, Papum Pare, Arunachal Pradesh, India.
* Corresponding author: pallabi2008rgu@gmail.com

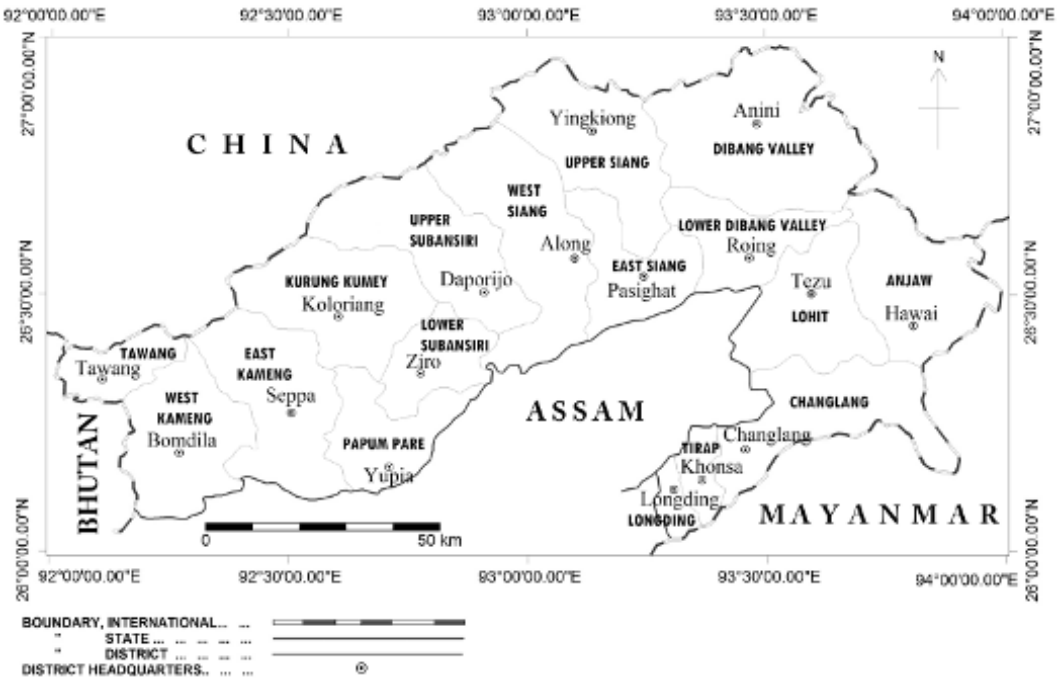


Fig. 12.1 Map of Arunachal Pradesh showing different districts and its headquarters.

biological diversity. Such an unparalleled occurrence of life form can attributed to the peculiar location of the state which is at the junction of Paleo Arctic, Indo-Chinese and Indo-Myanmar Biogeographical region (Taktajan 1996).

The vegetation type of the state have been classified into different forest types by various workers (Champion and Seth 1968, Haridasan and Rao 1985–1987) who classified the vegetation type of North East India. Later, Haridasan made the detailed study and classified the forest type of Arunachal Pradesh into four broad vegetation types which are further sub-classified based on prevailing plants (Haridasan 2001). The forests of Arunachal Pradesh are a powerhouse of a rich collection of medicinal plants, foods, aromatic and other economically useful plants comprising almost all the vegetation types of the country. The state harbors over 7000–8000 flowering plants (Hussain and Hore 2008) starting from the foothills to the snowline alpine region. Till date, over 800 medicinal plants reported from wild habitat have been found used in folk medicine while many others are still under exploration (Srivastava and Adi community 2009, Tag et al. 2014). Owing to its rich biodiversity new records or species are still reported by botanists (Banik et al. 2003). However with the growing advent of science and research yielding high throughput results researchers have come to the conclusion that certain plants that have a potent biological effect viz. antibacterial, antifungal, anti-inflammatory, antipyretic, etc., are a result of certain secondary metabolites that are prevalent among the plant family. However they may be or may not be directly correlated but some kind of association does exist which renders these plants vital for mankind. It is only since the late 20th century that secondary metabolites have been clearly recognized as having important functions in plants. Many thousands of secondary metabolites have been isolated from plants of late, and many of them have shown powerful physiological effects in humans and as such are used as medicines. Some of the wild medicinal plants viz., *Hedyotis scandens*, *Lasia spinosa*, *Elatostema sublaxum*, *Ficus semicordata*, *Musa balbisiana*, *Bryonia grandis*, *Artemisia nilagirica*, *Dendrocnide sinuata*, *Clerodendrum colebrookianum, Zanthoxylum armatum*, *Acorus calamus*, etc., are used in treating common diseases and ailments like dysentery, diarrhea, gastritis disorder, constipation, menstrual problem, toothache, headache, stomach ache, fever, typhoid, pneumonia, allergy, urination problem, jaundice, diabetes, eye-infection, high blood pressure, kidney stone, anti-dandruff, anti-lice in the hair and other uses.

Keeping in mind the broad prospect of the rich biodiversity of Arunachal Himalayas towards the global scenario, the aim of this chapter is to provide an insight of a wide range of secondary metabolites that are present in this biodiversity rich zone of Northeast India. The region is enriched with different medicinally important plants across different agro-climatic zone. Here we focus on some commonly available medicinal plants of this region with a diverse range of secondary metabolites to have an outlook of future prospect of such rich zonal area harboring some endemic, rare and endangered flora species of the world.

Classification of secondary metabolites

Secondary metabolites largely fall into three classes of compounds: alkaloids, terpenoids, and phenolics. However, these classes of compounds also include primary metabolites, so whether a compound is a primary or secondary metabolite is a distinction based not only on its chemical structure but also on its function and distribution within the plant kingdom.

Alkaloids

Alkaloids are a large group of nitrogen-containing compounds, examples of which are known to occur in approximately 20% of all flowering plants (Secondary metabolites – Knowledge Encyclopaedia 2016). Closely related plant species often contain alkaloids of related chemical structure. The primary metabolites from which they are derived include amino acids such as tryptophan, tyrosine, and lysine. Alkaloid biosynthetic pathways can be long, and many alkaloids have correspondingly complex chemical structures. Alkaloids accumulate in plant organs such as leaves or fruits and are ingested by animals that consume those plant parts. Many alkaloids are extremely toxic, especially to mammals, and act as potent nerve poisons, enzyme inhibitors, or membrane transport inhibitors. In addition to being toxic, many alkaloids are also bitter or otherwise foul-tasting. Therefore, the presence of alkaloids and other toxic secondary metabolites can serve as a deterrent to animals, who learn to avoid eating such plants. Sometimes domesticated animals that have not previously been exposed to alkaloid-containing plants do not have acquired avoidance mechanisms, and as such they become poisoned.

For example, *Senecio vulgaris*, often known by the common name 'groundsel' contains Pyrrolizidine Alkaloids (PAs) one of which is named '*senecionine*' which has resulted in many recorded cases of livestock fatalities due to liver failure. More frequently, over time, natural selection has resulted in animals developing biochemical mechanisms or behavioral traits that lead to avoidance of alkaloid-containing plants. Sometimes although a less common phenomenon, animals may evolve a mechanism for sequestering (storing) or breaking down a potentially toxic compound, thus '*disarming*' the plant. Plants have however with advent of time, acquired new capabilities to synthesize additional defense compounds to combat animals that have developed resistance to the original chemicals. This type of an '*arms race*' is a form of co-evolution and may help to account for the incredible abundance of secondary metabolites in flowering plants.

Medicinal Alkaloids

Many potentially toxic plant-derived alkaloids have medicinal properties, as long as they are carefully administered with regular dosage levels (Fig. 12.2). Alkaloids with important medicinal uses include '*morphine*' and '*codeine*' from the opium poppy (*Papaver somniferum*) and cocaine from the coca plant (*Erythroxylum* sp.). These alkaloids act on the nervous system and are used as painkillers. '*Atropine*' from the deadly 'nightshade plant' (*Atropa belladonna*), also acts on the nervous system and is used in anaesthesia and ophthalmology.

Vincristine and *vinblastine* from the 'periwinkle plant' (*Vinca minor*) are inhibitors of cell division and are used to treat cancers of the blood and lymphatic systems. Quinine from the bark of the 'cinchona tree' (*Cinchona officinalis*) is toxic to the *Plasmodium* parasite, which causes malaria, and has long been used in tropical and subtropical regions of the world.

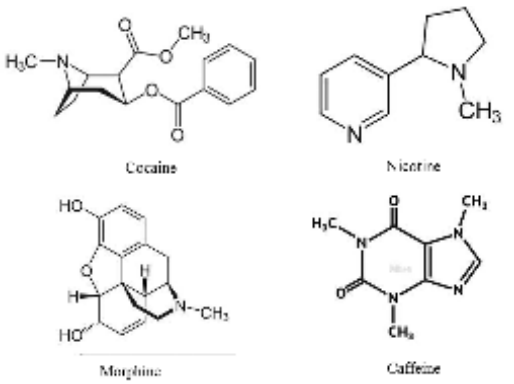


Fig. 12.2 Some examples of alkaloids, a diverse group of secondary metabolites that contain nitrogen (source: Taiz and Zeiger 2010).

Other alkaloids are used as stimulants, including *caffeine*, present in coffee (*Coffea* sp.), tea (*Camellia sinensis*), and cola plants (*Cola* sp.), and nicotine, which is present in tobacco (*Nicotiana tabacum*). Nicotine preparations are, paradoxically, also used as an aid in smoking cessation. Nicotine is also a very potent insecticide. Ground-up tobacco leaves were used for insect control for many years, but this practice was superseded by the use of special formulations of nicotine. More recently the use of nicotine as an insecticide has been discouraged because of its toxicity to humans.

Terpenoids

The terpenoids sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from the assembly of five-carbon isoprene units with different modifications. They are derived from acetyl CoA or from intermediates in glycolysis (Cornelia and Ludger 2012). They are classified by their number of five-carbon isoprenoid units. Monoterpenes (containing two C_5-units) are exemplified by the fragrant oils (such as menthol) contained in the leaves of members of the mint (*Lamiaceae*) family. In addition to giving these plants their characteristic taste and odor, these volatile oils have insect-repellent qualities (Fig. 12.3).

The *pyrethroids*, which are monoterpene esters from the flowers of '*chrysanthemum*' and related species, are commercially used as insecticides. In spite of being biodegradable and nontoxic to mammals, they fatally affect the nervous systems of insects, including humans.

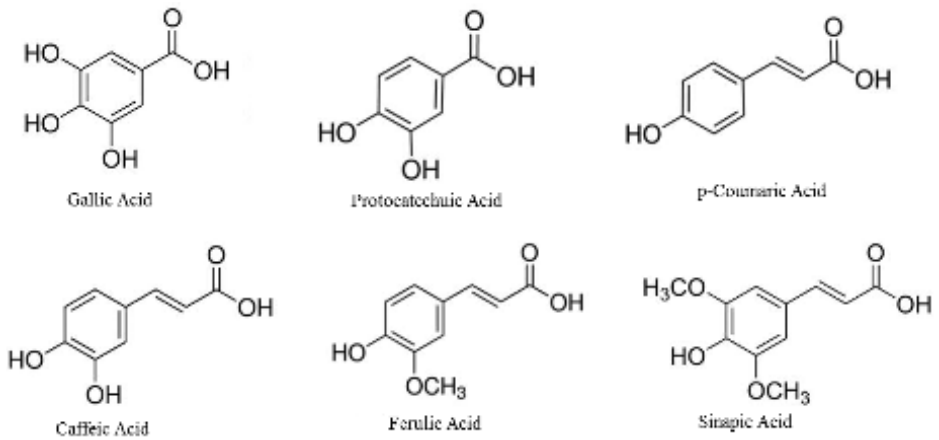


Fig. 12.3 Structures of limonene (A) and menthol (B): two well-known monoterpenes that serve as defenses against insects and other organisms (source: Taiz and Zeiger 2010).

Diterpenes are formed from four C_5-units. *Paclitaxel* (commonly known by the brand name *Taxol*), a diterpene found in bark of the genus '*Taxus*', is a potent inhibitor of cell division in animals. At the end of the 20th century, *paclitaxel* was developed as a powerful new chemotherapeutic treatment for people with solid tumors, such as ovarian cancer patients.

Triterpenoids (formed from six C_5 units) comprise the plant steroids, some of which act as plant hormones. These also can protect plants from insect attack, though their mode of action is quite different from that of the pyrethroids. Examples include '*phytoecdysones*', a group of plant sterols that resemble insect molting hormones. When ingested in excess it can disrupt the normal molting cycle with often lethal consequences to the insect.

Tetraterpenoids (eight C_5 units) include important pigments such as β-*carotene*, which is a precursor of vitamin A, and *lycopene*, which gives tomatoes (*Solanum lycopersicum*) their red color. Rather than functioning in plant defense, the colored pigments that accumulate in ripening fruits can serve as attractants to animals, which actually aid the plant in seed dispersal.

The polyterpenes are polymers that may contain several thousand isoprenoid units. *Rubber*, a polyterpene in the latex of rubber trees (*Hevea brasiliensis*) that probably aids in wound healing in the plant, is also very important for the manufacture of tires and other products.

Phenolic compounds

Phenolic compounds are defined by the presence of one or more aromatic rings bearing a hydroxyl functional group. Many are synthesized from the amino acid phenylalanine. They are important for the quality of plant based foods and are responsible for imparting color of red fruits, juices and wines and substrates for enzymatic browning, and are also involved in flavor properties. Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, etc.

Simple phenolic compounds, such as '*salicylic acid*' can be important in defence against attack of fungal pathogens. Its concentration increases in the leaves of certain plants in response to fungal attack and enables the plant to mount a complex defense response.

Interestingly '*aspirin*' a derivative of salicylic acid, is routinely used in humans to reduce inflammation, pain, and fever. Other phenolic compounds, called '*isoflavones*' are synthesized rapidly in plants of the legume (*Fabaceae*) family when they are attacked by bacterial or fungal pathogens, and they have strong antimicrobial activity.

One of the complex phenolic macromolecule, '*Lignin*' is laid down in plant secondary cell walls and is the main component of wood. It is a very important structural molecule in all woody plants, allowing them to achieve height, girth, and longevity. It is also valuable for plant defense: Plant parts containing cells with lignified walls are much less palatable to insects and other animals than are non-woody plants and are much less easily digested by fungal enzymes than plant parts that contain only cells with primary cellulose walls.

Other function as attractants. '*Anthocyanins*' and '*Anthocyanidins*' are phenolic pigments that impart pink and purple colors to flowers and fruits. This pigmentation attracts insects and other animals that move between individual plants and lead to pollination and fruit dispersal. Often the plant pigment and the pollinator's visual systems are well matched: Plants with red flowers attract birds and mammals because these animals possess the correct photoreceptors to see red pigments.

Phenolic compounds: antioxidant activity, occurrence, and potential uses

Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds possess an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. Flavonoids, which bear the (C_6–C_3–C_6) structure, account for more than half of the over eight thousand different phenolic compounds. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and

the nature of substitutions on the aromatic rings. Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet (Balakumbahan et al. 2010).

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants (Taiz and Zeiger 2010). These compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Alasalvar et al. 2001), besides contributing towards the color and sensory characteristics of fruits and vegetables (Benavente-Garcia et al. 1997).

Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio-protective and vasodilatory effects (Samman et al. 1998, Middleton et al. 2000, Puupponen Pimia et al. 2001, Claudine et al. 2005). Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (Harborne 1989, Parr and Bolwell 2000).

Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds. Despite this structural diversity, the groups of compounds are often referred to as '*polyphenols*'. Most naturally occurring phenolic compounds are present as conjugates with mono and polysaccharides, linked to one or more of the phenolic groups, and may also occur as functional derivatives such as esters and methyl esters (Shahidi and Naczk 1995, Harborne et al. 1999, King and Young 1999). Though such structural diversity results in a wide range of phenolic compounds that occur in nature, phenolic compounds can basically be categorized into several classes as shown in Table 12.1 (Shahidi and Naczk 1995, King and Young 1999). Among these, phenolic acids, flavonoids and tannins are regarded as the main dietary phenolic compounds (Bhom 1998).

Phenolic acids consist of two subgroups, i.e., the hydroxybenzoic and hydroxycinnamic acids (Fig. 12.4). Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids, which in common have the (C_6–C_1) structure. Hydroxycinnamic acids, on the other hand, are aromatic compounds with a three-carbon side chain (C_6–C_3), with *caffeic acid*, *ferulic acid*, *p-coumaric acid* and *sinapic acid* being the most common.

Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds. Flavonoids are low molecular weight compounds, consisting of 15 carbon atoms, arranged in a (C_6–C_3–C_6) configuration. Essentially the structure consists of two aromatic rings A and B, joined by a three carbon bridge, usually in the form of a heterocyclic ring C. The aromatic ring A is derived from the acetate/malonate pathway, while ring B is derived from phenylalanine through the shikimate pathway (Hollman and Katan 1999, Merken and Beecher 2000). Variations in substitution patterns to ring C result in the major flavonoid classes, i.e., *flavonols, flavones, flavanones, flavanols* (or

Table 12.1 Classes of phenolic compounds in plants.

Class	**Structure**
Simple phenolics, benzoquinones	C_6
Hydroxybenzoic acids	(C_6–C_1)
Acethophenones, phenylacetic acids	(C_6–C_2)
Hydroxycinnamic acids, phenylpropanoids (coumarins, isocoumarins, chromones, chromenes)	(C_6–C_3)
Napthoquinones	(C_6–C_4)
Xanthones	(C_6–C_1–C_6)
Stilbenes, anthraquinones	(C_6–C_2–C_6)
Flavonoids, isoflavonoids	(C_6–C_3–C_6)
Lignans, neolignans	(C_6–C_3)$_2$
Biflavonoids	(C_6–C_3–C_6)$_2$
Lignins	(C_6–C_3)n
Condensed tannins (proanthocyanidins or flavolans)	(C_6–C_3–C_6)n

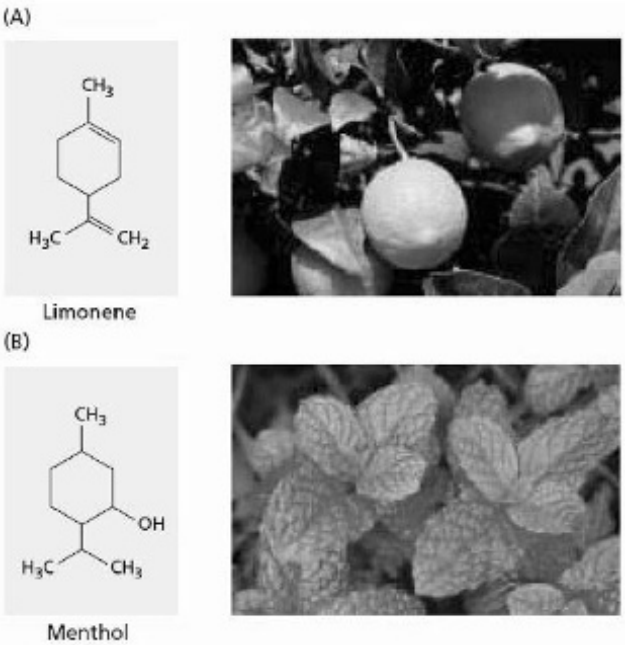


Fig. 12.4 Examples of some hydroxybenzoic and hydroxycinnamic acids.

catechins), *isoflavones, flavanonols,* and *anthocyanidins* (Pietta 2000), of which *flavones* and *flavonols* occur most widely and are structurally diverse. Substitutions to rings A and B give rise to the different compounds within each class of flavonoids. These substitutions may include oxygenation, alkylation, glycosylation, acylation, and sulfation (Pietta 2000).

Tannins, the relatively high molecular weight compounds which constitute the third important group of phenolics may be subdivided into hydrolysable tannins and condensed tannins (Porter 1989). The former are esters of gallic acid (gallo- and ellagi-tannins), while the latter (also known as proanthocyanidins) are polymers of polyhydroxyflavan-3-ol monomers (Porter 1989). Some common medicinal plants of Arunachal Pradesh with their photographs (Fig. 12.5) and types of secondary metabolites are given in below (Table 12.2).

Understanding relationships between secondary metabolites of plants and its associated medicinal properties for the future generation

Since last two decades, significant ethnobotanical works have been published from this region (Tiwari et al. 1976, Thothathri and Pal 1987, Kar 2004, Tag and Das 2004, Kala 2005, Tag et al. 2005, Angami et al. 2006, Das and Tag 2006, Ramashankar and Rawat 2008, Goswami et al. 2009, Tiwari et al. 2009, Doley et al. 2010, 2014, Kagyung et al. 2010, Rethy et al. 2010, Sarmah and Arunachalam 2010, Srivastava et al. 2010, Srivastava and Nyishi Community 2010, Jeri et al. 2011, Namsa et al. 2011, Nimachow et al. 2011, 2012, Shrivastava et al. 2012, Dutta and Dutta 2013, Maiti et al. 2013, Monlai et al. 2013, Payum et al. 2013, Singh and Singh 2013, Yakang et al. 2013, Boko and Narsimhan 2014, Perme et al. 2015, Purwianingsih et al. 2015, Murtem and Chaudhry 2016, Tsering 2016, Tsering et al. 2017, Tripathi et al. 2017). However, such studies have also revealed that many tribal groups of the eastern Himalayan zone including Arunachal Himalayas of India are either under-explored or unexplored with regards to their ethnobotanical and ethno-medicinal research works due to communication bottleneck and topographical disadvantage. Such unexplored plants, are rich repositories of wide range of secondary metabolites (Table 12.2). It is obvious that these secondary metabolites are useful to plants but can be catabolized. Several of these products have shown to exert a profound physiological effect on the mammalian system and therefore are termed bioactive compounds. An intricate understanding of relationships between secondary

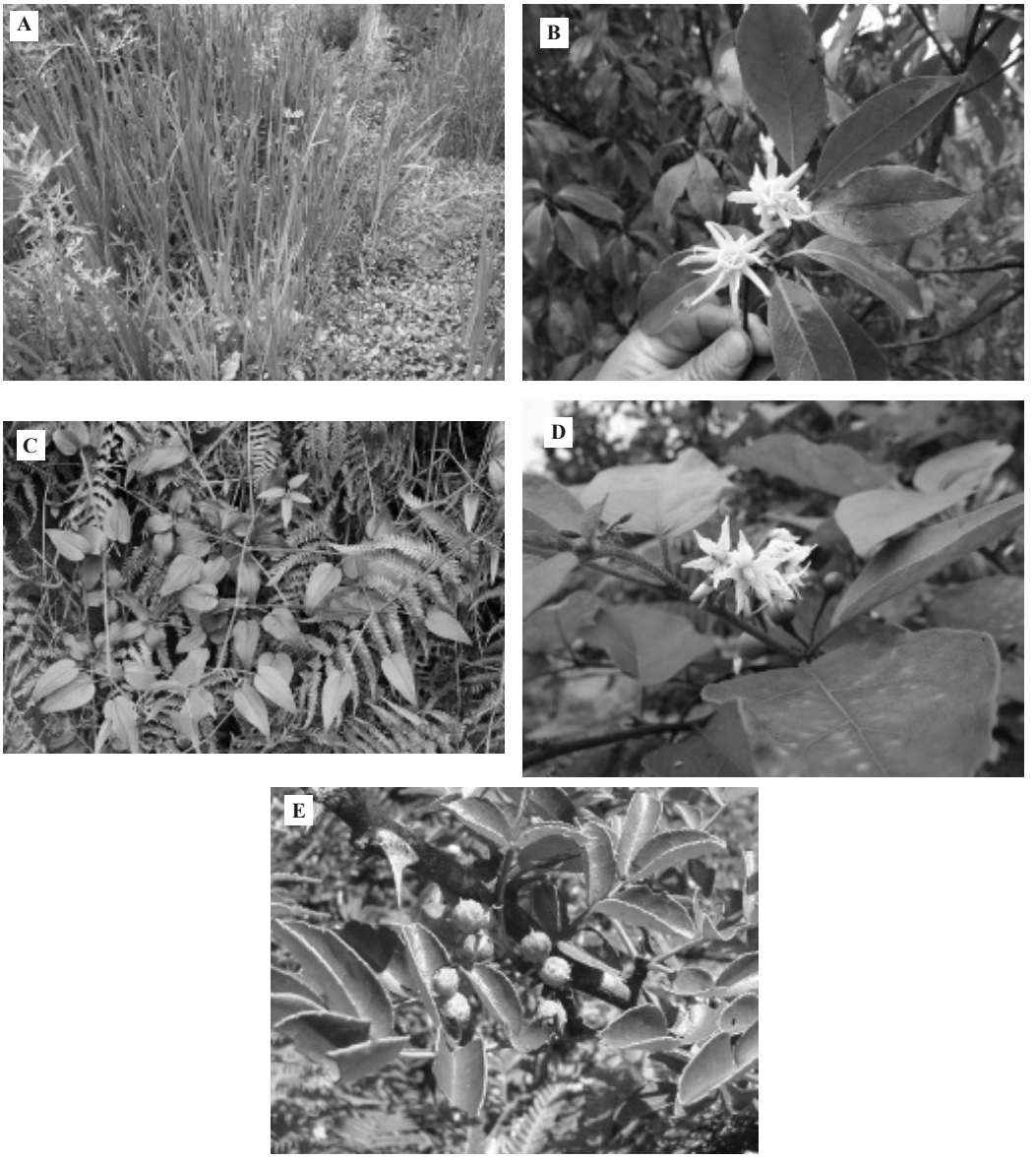


Fig. 12.5 Some representative ethnomedicinal plants in Arunachal Pradesh (A) *Acorus calamus* L. (B). *Illicium griffithii* Hook.f. & Thomson (C) *Rubia cordifolia* L. (D) *Solanum torvum* Sw. (E) *Zanthoxylum armatum* DC. (Photographs taken by Dr. Hui Tag during his several field surveys.)

metabolites of plants and its associated medicinal property is henceforth necessary to validate such rich repositories of energy, food, shelter and medicinal wealth in Arunachal Pradesh that would serve as a pioneer for future states of the country as well as other nations. It's high time that we focus our attention to the complex relationship between the secondary metabolites and its associated medicinal property of these plants rather than viewing each of them in an isolated mode, because all are intermingled together and have strong correlation between them. Such correlated studies could be the answer for the current global concern of environmental issues, biodiversity degradation, conservation of bio-resources, hazardous diseases, etc. Therefore keeping in mind the broad prospect of such secondary metabolites that are present in the rich biodiversity of ethno-medicinal plants of Arunachal Pradesh, our future target must be to document and validate such traditionally useful bio-resources and establish the relationship between the secondary metabolites and its related bioactive compounds. Moreover extinction of traditional knowledge has been

Table 12.2 List of some common ethno-medicinal plants of Arunachal Pradesh with types of secondary metabolites.

Sl No.	Name of the plant	Family	Traditional use	Active compounds	Type of secondary metabolites
1.	*Aconitum ferox* Wall. ex Ser.	Ranunculaceae	Paste of tuber is applied at the tip of the arrows for hunting animals (Tag and Das 2004).	Aconitin, Pseudo-aconitin, Bikh-aconitine, Diacetyl-pseudaconitin, Aconine (Giri et al. 1997)	Diterpene alkaloids
2.	*Acorus calamus* L.	Acoraceae	Rhizome is chewed for toothache, inhaled for congestion; used as brain tonic, coolant and drug for colic and as a remedy for digestive disorders (Balakumbahan et al. 2010).	β-asarone (McGaw et al. 2002).	Ether
3.	*Ageratum conyzoides* (L.) L.	Asteraceae	Paste of entire plant is applied on wounds for healing and blood clotting; plant juice is applied twice daily in red eye (conjuctivitis); plants are pounded and made into pills, the size of a pea and taken to cure blood dysentery; whole plant also used as fish poison (Srivastava and Adi Community 2009).	Stigmasterol and β-sitosterol. (Kamboj and Saluja 2011).	Sterols
4.	*Andrographis paniculata* (Burm.f.) Nees	Acanthaceae	Crushed leaves mixed in warm water is administered for the treatment of malaria and cough (Sen et al. 2008).	Andrographolide (Pholphana et al. 2004).	Labdane diterpenoid
5.	*Bryophyllum pinnatum* (Lam.) Oken	Crassulaceae	Juice of leaves is applied on burnt parts of the body (Boko and Narsimhan 2014).	Bryotoxin A, B, C, 4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxy benzoic acid (Kamboj and Saluja 2009).	Bufadienolides flavonoids, Polyphenols, Triterpenoids
6.	*Clerodendrum glandulosum* Lindl.	Verbenaceae	Used for the treatment of malarial parasite (Wangpan et al. 2016).	1,3-dimethyl-5-(2,5,13,17-tetramethyl octadeca-6,16-dien-8-yl) benzene, β–sitosterol, Clerodolone (Joshi et al. 1979).	Triterpenoid, Sterols
7.	*Coptis teeta* Wall.	Ranunculaceae	Rhizome used in eye diseases and as a good appetizer, curing the digestive system. Anti-inflammatory and is useful in skin disorders (Bhattee and Beniwal 1988).	Berberine, Coptine (Xu et al. 2010).	Alkaloids
8.	*Dillenia indica* L.	Dilleniaceae	Fruits and fleshy calyx with little salt are taken raw or boiled and is used for treating stomachache (Khongsai et al. 2011).	Betulinic acid (Kumar et al. 2010).	Pentacyclic triterpenoid

9.	*Dioscorea floribunda* M. Martens & Galeotti	Dioscoreaceae	Tubers powders with water used in ulcer, malaria, headache, fever (Ramashankar and Rawat 2008).	Diosbulbin A, B, C, D, E Diosgeninan (Hoyer et al. 1975).	Saponins Alkaloid
10.	*Drymaria cordata* (L.) Willd. ex Schult.	Caryophyllaceae	Leaf paste is applied on forehead to get relief from headache; fresh whole plant is mixed with *Psidium guajava* and is taken during gastritis (Kagyung et al. 2010).	Drymaritin , C-Glycoside Flavanoid (Hsieh et al. 2004).	Alkaloids, Flavonoids,
11.	*Illicium griffithii* Hook.f. & Thomson	Schisandraceae	Used as medicine to cure abdominal pain, cough, dyspepsia, food poisoning, vomiting, toothache and sinusitis. Fruits are also used as incense, flavoring tea, to increase the potency of alcohol, preparing butter salted tea or sugar tea for sweet fragrance. It is also used as an antifungal agent and food preservative (Murtem and Chaudhry 2016).	Shikimic acid (Qinh et al. 2016).	Cyclohexane Carboxylic acid, Sesquiterpene lactone
12.	*Oroxylum indicum* (L.) Kurz.	Bignoniaceae	Stem bark powder used in breast cancer (Tripathi et al. 2017).	Oroxylin, Baicalein, Stigmast-7-en-3-ol (Luitel et al. 2010).	Flavones, Sterols
13.	*Paedaria foetida* L.	Rubiaceae	Boiled leaves & twigs are taken with rice as a vegetable to cure diarrhea & dysentery, the paste of the leaves applied to skin diseases; also useful for curing stomachache & gastric indigestion (Chanda et al. 2013).	Linalool (Wong and Tan 1994).	Monoterpenes
14.	*Piper longum* L.	Piperaceae	Leaf after rubbing with mustard oil and warming over burning charcoal is applied to belly during stomach ache of children (Perme et al. 2015).	Pipernonaline, Piperoctadecalidine, Piperine (Park et al. 2002).	Alkaloids
15.	*Rubia cordifolia* L.	Rubiaceae	Root used as tonic, astringent; stem used as antidote for cobra bite and scorpion sting, used as a blood clotting agent (Hussain and Hore, 2008).	Cordifoliol Cordifodiol, Rubiacordone A(1), Purpurin (Li et al, 2009).	Anthraquinone
16.	*Solanum torvum* Sw.	Solanaceae	Crushed fruits are applied to gums to get relief from gum infection and toothache (Namsa et al. 2011).	spirostanol saponins, spirostanol glycosides (Lu et al. 2008).	Saponins, Glycoside conjugates
17.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Ground and drunk with water; fruits are directly consumed, helps in curing cold, cough & fever (Khongsai et al. 2011).	Termilignan, Thannilignan (Valsaraj et al. 1997).	Lignans

Table 12.2 contd....

...Table 12.2 contd.

Sl No.	Name of the plant	Family	Traditional use	Active compounds	Type of secondary metabolites
18.	*Tinospora sinensis* (Lour.) Merr.	Menispermaceae	Stem decoction for strengthening of bones (Srivastava and Nyishi Community 2010).	Tinosinaside, Diosgenin, Cordioside (Srinivasan et al. 2008).	Steroids, Glycosides, Carbohydrates
19.	*Zanthoxylum armatum* DC.	Rutaceae	Crude pounded fruits are used as fish poison; tender leaves are used as vegetable; infusion of seeds mixed with *Allium sativum* and little salt is prescribed in case of stomach bloating (Khongsai et al. 2011).	Armatamide, Asarinin, Fargesin (Kalia et al. 1999).	Amides, Lignans
20.	*Zingiber officinale* Roscoe	Zingiberaceae	Used as edible; Rhizome is used for different religious purpose (Boko and Narsimhan 2014).	β-sitosterol, Palmitate, Isovanillin, Glycol mono-palmitate (Bao et al. 2010).	Sterols, Phenolic aldehyde

witnessed in many parts of the state particularly districts of Tawang and West Kameng region because of rapid changes and developmental process in an effort to make it a global tourist hotspot (Tsering 2016). Therefore there is also a need to protect such medicinally important plants which can be only done by imparting knowledge of different conservation strategies in the public domain especially to the villagers through conducting various awareness programs. They also need to be involved in food biodiversity based natural resource conservation by demonstrating rural biotechnology tools and methods for effective conservation and sustainable management of these mountain plant bio-resources for rural livelihood security and the future generations of mankind. Such an awareness campaign would also ensure significance of such valuable botanical resources in current demand and supply trends of wild edible plants in the local and regional markets.

Conclusion

Thus, such rare and endemic medicinal plants of Arunachal Pradesh need conservation attention in the present decade to prevent rampant illegal collection from the wild habitat as well as for obtaining goods and services and ecological stability. Since local communities have deep faith in traditional methods of herbal treatment, they are still using some plants in treatment of several human ailments some of which are not recorded in any medicinal literatures including Ayurveda. Such plants need to be explored judiciously as well as protected from habitat destruction and other possible threats. Plant tissue culture techniques viz. *in vitro* organ culture, cell suspension cultures and callus culture can be effective in curbing the cost of production in the process of extraction, isolation and purification of secondary metabolites extracted directly from these plants (Purwianingsih 2015). Development of appropriate agro-technology research is urgently required to minimize wild collection and that would ensure *ex situ* conservation and sustainable medicinal and commercial uses making this state undoubtedly the living encyclopaedia and cultural refugia in biodiversity conservation of the eastern Himalayan Region.

References

Alasalvar, C., Grigor, J.M., Zhang, D., Quantick, P.C. and Shahidi, F. 2001. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. J. Agric. Food Chem. 49: 1410–1416.

Angami, A., Gajurel, P.R., Rethy, P., Singh, B. and Kalita, S.K. 2006. Status and potential of wild edible plants of Arunachal Pradesh. Indian J. Trad. Knowl. 5(4): 541–550.

Anonymous. Forest Statistics of Arunachal Pradesh. 2008. Department of Environment and Forest, Govt. of Arunachal Pradesh Itanagar, pp. 3–30.

Balakumbahan, R., Rajamani, K. and Kumanan, K. 2010. *Acorus calamus*: an overview. J. Med. Plant Res. 4(25): 2740–2745.

Banik, A., Sarmah, A. and Muthu, J. 2003. *Agapetes neriifolia* (King and Prain) Airy Ashaw var. minor (King and Prain) Airy Shaw – New record for India. Bull. Bot. Surv. India, Calcutta 45(1-4): 213–216.

Bao, L., Deng, A., Li, Z., Du, G. and Qin, H. 2010. Chemical constituents of rhizomes of *Zingiber officinale*. China J. Chin. Mat. Med. 35(5): 598–601.

Benavente-Garcia, O., Castillo, J., Marin, F.R., Ortuno, A. and Del, Rio, J.A. 1997. Uses and properties of *citrus* flavonoids. J. Agric. Food Chem. 45: 4505–4515.

Bhattee, S.S. and Beniwal, B.S. 1988. *Coptis teeta* Wall—an important and valuable medicinal plant of Arunachal Pradesh and its cultivation. Indian For. 144(5): 251–260.

Bhom, B.A. 1998. Introduction to Flavonoids. Harwood Academic Publishers, Amsterdam.

Boko, N. and Narsimhan, D. 2014. Rapid survey of plants used by Adi tribe of Bosing-Banggo, East Siang District, Arunachal Pradesh, India. Pleione 8(2): 271–282.

Champion, H.G. and Seth, S.K. 1968. A Revised Survey of the Forest Types of India. Manager of Publication, New Delhi, India.

Chanda, S., Sarethy, I.P. and De, B. 2013. *Paederia foetida*—a promising ethno-medicinal tribal plant of northeastern India. J. For. Res. 24(4): 801.

Claudine, M., Mazur, A. and Scalbert, A. 2005. Polyphenols and prevention of cardiovascular diseases. Curr. Opin. Lipidol. 16: 77–84.

Cornelia, C. and Ludger, B. 2012. Phytoalexins of the pyrinae: biphenyls and dibenzofurans. Beilstein J. Org. Chem. 8: 613–620.

Das, A.K. and Tag, H. 2006. Ethnomedicinal studies of the Khamti tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 5(3): 317–322.

Doley, B., Gajurel, P.R., Rethy, P., Singh, B. and Hazarika, H. 2010. Ethnomedicinal uses of different species of *Cinnamomum schaeffer* (Lauraceae) by ethnic community in Arunachal Pradesh, India. Pleione 3(1): 9–12.

Doley, B., Gajurel, P.R., Rethy, P. and Buragohain, R. 2014. Uses of trees as medicine by the ethnic communities of Arunachal Pradesh, India. J. Med. Pl. Res. 8(24): 857–863.

Dutta, R. and Dutta, A. 2013. Bheta fishing—a traditional community fishing practice of nocte tribe of tirap district, Arunachal Pradesh. Indian J. Trad. Knowl. 12(1): 162 – 165.

Giri, A., Banerjee, S., Ahuja, P.S. and Giri, C.C. 1997. Production of hairy roots in *Aconitum heterophyllum* Wall. using *Agrobacterium rhizogenes*. *In Vitro* Cell Dev. Biol. - Plant 33(4): 280–284.

Goswami, P., Soki, D., Jaishi, A., Das, M. and Sarma, H.N. 2009. Traditional healthcare practices among the tagin tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 8(1): 127–130.

Harborne, J.B., Baxter, H. and Moss, G.P. (eds.). 1999. Photochemical Dictionary the Handbook of Bioactive Compound from Plants (2)1999. Taylor & Francis, London.

Harborne, J.B. 1989. General procedure and measurement of total Phenolics. Methods in Plant Biochemistry. Volume 1. Plant phenolics. London Academic Press Ltd., 1989, pp 1–28.

Haridasan, K. and Rao, R.R. (1985–87). Forest Flora of Meghalaya. Vol. I&II. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Haridasan, K. 2001. Ethnobotanical studies in Arunachal Pradesh—A status Report. pp. 154–167. *In*: Gian Singh, Singh, H.B. and Mukherjee, T.K. (eds.). Ethnomedicine of North-East India, NISCIR (CSIR), New Delhi.

Hollman, P.C.H. and Katan, M.B. 1999. Dietary flavonoids: intake, health effects and bioavailability. Food Chem. Toxicol. 37: 937–942.

Hoyer, G.A., Sucrow, W. and Winkler, D. 1975. Diosgenin saponins from *Dioscorea floribunda*. Phytochemistry 14(2): 539–542.

Hussain, S. and Hore, D.K. 2008. Collection and conservation of major medicinal plants of Arunachal Pradesh. Indian For. 134(12): 1663–1679.

Jeri, L., Tag, H., Tsering, J., Kalita, P., Mingki, T. and Das, A.K. 2011. Ethnobotanical Investigation of Edible and Medicinal Plants in Pakke Wildlife Sanctuary of East Kameng District in Arunachal Pradesh, India. Pleione 5(1): 83–90.

Joshi, K.C., Singh, P. and Mehra, A. 1979. Chemical investigation of the roots of different *Clerodendron* species. Planta Med. 37(9): 64–66.

Kagyung, R., Gajurel, P.R., Rethy, P. and Singh, B. 2010. Ethnomedicinal plants used for gastro-intestinal diseases by Adi tribes of dehang-debang biosphere reserve in Arunachal Pradesh. Indian J. Trad. Knowl. 9(3): 496–501.

Kala, C.P. 2005. Ethnomedicinal Botany of Apatani in the Eastern Himalayan Region of India. J. Ethnobio. Ethnomed. 1:11.

Kalia, N.K., Singh, B. and Sood, R.P. 1999. A new amide from *Zanthoxylum armatum*. J. Nat. Prod. 62(2): 311–312.

Kamboj, A. and Saluja, A.K. 2009. *Bryophyllum pinnatum* (Lam.) Kurz.: Phytochemical and pharmacological profile: A review. Ph. Cog. Rev. 3: 364–74.

Kamboj, A. and Saluja, A.K. 2011. Isolation of Stigmasterol and β Sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). Int. J. Pharm. Sci. 3(1): 94–96.

Kar, A. 2004. Common Wild Vegetables of Aka tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 3(3): 305–313.

Khongsai, M., Saikia, S.P and Kayang, H. 2011. Ethnomedicinal plants used by different tribes of Arunachal Pradesh. Indian J. Trad. Knowl. 10(3): 541–546.

King, A.M.Y. and Young, G. 1999. Characteristics and occurrence of phenolic phytochemicals. J. Am. Diet. Assoc. 99: 213–218.

Kumar, D., Mallick, S., Vedasiromoni, J.R. and Pal, B.C. 2010. Anti-leukemic activity of *Dillenia indica* L. fruit extract and quantification of betulinic acid by HPLC. Phytomedicine 17(6): 431–435.

Li, X., Liu, Z., Chen, Y., Wang, L.J., Zheng, Y.N., Sun, G.Z. and Ruan, C.C. 2009. Rubiacordone A: a new anthraquinone glycoside from the roots of *Rubia cordifolia*. Molecules 14(1): 566–572.

Lu, Yuanyuan, Luo, J., Xu, D., Huang, X. and Kong, L. 2008. Characterization of spirostanol saponins in *Solanum torvum* by high-performance liquid chromatography/evaporative light scattering detector/electrospray ionization with multi-stage tandem mass spectrometry. Rapid Commun. Mass Sp. 22(16): 2447–2452.

Luitel, H.N., Rajbhandari, M., Kalauni, S.K., Awale, S., Masuda, K. and Gewali, M.B. 2010. Chemical constituents from *Oroxylum indicum* (L.) Kurz of Nepalese Origin. Sci. World (8): 8–21.

Maiti, S., Chakravaty, P., Garai, S., Bandyopadhyay, S. and Chouhan, V.S. 2013. Ethno veterinary practices for ephemeral fever of yak: A participatory assessment by the Monpa tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 12(1): 36–39.

McGaw, L.J., Jäger, A.K. and Staden, J.V. 2002. Isolation of β-asarone, an antibacterial and anthelmintic compound, from *Acorus calamus* in South Africa. S. Afr. J. Bot. 68(1): 31–35.

Merken, H.M. and Beecher, G.R. 2000. Measurement of food flavonoids by high-performance liquid chromatography: a review. J. Agr. Food Chem. 48: 577–599.

Middleton, E., Kandaswami, C. and Theoharides, T.C. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52: 673–751.

Monlai, S., Lalramnghinglova, H. and Arunachalam, A. 2013. Traditional Tai-Khampti medicinal plants to cure fracture bones. Pleione 7(2): 469–472.

Murtem, G. and Chaudhry, P. 2016. An ethnobotanical note on wild edible plants of Upper Eastern Himalaya, India. Brazilian J. Biol. Sci. 3(5): 63–81.

Namsa, N.D., Mandal, M., Tangjang, S. and Mandal, S.C. 2011. Ethnobotany of the Monpa ethnic group of Arunachal Pradesh. J. Ethnobio. Ethnomed. 7: 31. 1746-4269-7-31.

Nimachow, G., Joshi, R.C. and Dai, O. 2011. Role of indigenous knowledge system in conservation of forest resources—a case study of the Aka tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 10(2): 276–280.

Nimasow, G., Ringu, N. and Nimasow, O.D. 2012. Ethnomedicinal Knowledge among the Adi tribes of Lower Dibang Valley, Arunachal Pradesh, India. Int. Res. J. Pharm. 3(6): 223–229.

Park, B.S., Lee, S.E., Choi, W.S., Jeong, C.Y., Song, C. and Cho, K.Y. 2002. Insecticidal and acaricidal activity of pipernonaline and piperoctadecalidine derived from dried fruits of *Piper longum* L. Crop Prot. 21: 249–251.

Parr, A.J. and Bolwell, G.P. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci. Food Agr. 80: 985–1012.

Payum, T., Das, A.K., Ramashankar, R., Tamuly, T. and Hazarika, M. 2013. Ethnobotany and Antioxidant determination of *Phoebe cooperiana* fruit—A highly utilized wild fruit in Arunachal Pradesh, India. Int. J. Pharm. Sci. Res. 4(8): 3196–3201.

Hsieh, P.W., Chang, F.R., Lee, K.H., Hwang, T.L., Chang, S.W. and Wu, Y.C. 2004. A new anti-HIV alkaloid, drymaritin, and a new C-glycoside flavonoid, diandraflavone, from *Drymaria cordata*. J. Nat. Prod. 67(7): 1175–1177.

Perme, N., Choudhury, S.N., Choudhury, R., Natung, T. and De, B. 2015. Medicinal plants in traditional use at Arunachal Pradesh, India. 2015. Int. J. Phytopharm. 5(5): 86–98.

Pholphana, N., Rangkadilok, N., Thongnest, S., Ruchirawat, S., Ruchirawat, M. and Satayavivad, J. 2004. Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm.f.) Nees. Phytochem. Anal. 15: 365–371.

Pietta, P.G. 2000. Flavonoids as antioxidants. J. Nat. Prod. 63: 1035–1042.

Porter, L.J. 1989. Tannins. pp. 389–419. *In*: Harborne, J.B. (ed.). Methods in Plant Biochemistry: Vol 1. Plant Phenolics. London academic press.

Purwianingsih, W., Febri, S. and Kusdianti. 2015. Formation Flavonoid Secondary Metabolites in Callus Culture of *Chrysanthemum cinerariefolium* as Alternative Provision Medicine. Proceedings of International Seminar on Mathematics, Science, and Computer Science Education (MSCEIS 2015).

Puupponen Pimia, R., Nohynek, L., Meier, C., Kahkonen, M., Heinonen, M. and Hopia, A. 2001. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. 90: 494–507.

Qinh, N.B., Dai, D.N, Than, B.V., Dung, V.T., Hang, V.T.T. and Ogunwande, I.A. 2016. Volatile Constituents of Three *Illicium* Plants. Rec. Nat. Prod. 10(6): 806–811.

Rethy, P., Singh, B., Kagyung, R. and Gajurel, P.R. 2010. Ethnobotanical studies of dehang-debang biosphere reserve of Arunachal Pradesh with special reference to Memba tribe. Indian J. Trad. Knowl. 9(1): 61–67.

Ramashankar, R. and Rawat, M.S. 2008. Medicinal plants used in traditional medicine in Lohit and Dibang valley districts of Arunachal Pradesh. Indian J. Trad. Knowl. 7(2): 288–295.

Samman, S., Lyons wall, P.M. and Cook, N.C. 1998. Flavonoids and coronary heart disease: Dietary perspectives. pp. 469–482. *In*: Rice-Evans, C.A. and Packer, L. (eds.). Flavonoids in Health and Disease. Marcel Dekker, New York.

Sarmah, R. and Arunachalam, A. 2010. Contribution of Non Timber Forest Products (NTFPS) to the Livelihood Economy of the People living in the Forest in Changlang district of Arunachal Pradesh, India. Indian J. Fund. Appl. Life Sci. 1(2): 157–169.

Secondary metabolites – Knowledge Encyclopedia" www. biology reference.com. Retrieved 2016-05-10.

Sen, P., Dollo, M., Dutta Choudhury, M. and Choudhury, D. 2008. Documentation of traditional herbal knowledge of Khamptis of Arunachal Pradesh. Indian J. Trad. Knowl. 7(3): 438–442.

Shahidi, F. and Naczk, M. 1995. Food phenolics: Sources, Chemistry, effects application. PA:Technomic Pub. Co., Inc., Lancaster.

Shrivastava, K., Greeshma, A.G. and Srivastava, B. 2012. Biotechnology in Traditional – methods of starter preparation for alcoholic beverages employed by different tribal communities of Arunachal Pradesh. Indian J. Trad. Knowl. 11(1): 194–201.

Singh, R.K. and Singh, A. 2013. Biodiversity and recipe contests: Innovative socioecological approaches to capture ecological knowledge and conserve biodiversity in Arunachal Pradesh. Indian J. Trad. Knowl. 12(2): 240–251.

Srinivasan, G.V., Unnikrishnan, K.P., Remashree, A.B. and Balachandran, I. 2008. HPLC Estimation of berberine in *Tinospora cordifolia* and *Tinospora sinensis*. India J. Pharm. Sci. 70(1): 96–99.

Srivastava, R.C. and Adi Community. 2009. Traditional knowledge of Adi tribe of Arunachal Pradesh on Plants. Indian J. Trad. Knowl. 8(2): 146–153.

Shrivastava, R.C. and Nyishi Community. 2010. Traditional knowledge of Nyishi (Daffla) tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 9(1): 26–37.

Srivastava, R.C., Singh, R.K., Apatani Community and Mukherjee, T.K. 2010. Indigenous biodiversity of Apatani plateau: Learning on bioculture knowledge of Apatani tribe of Arunachal Pradesh for sustainable livelihood. Indian J. Trad. Knowl. 9(3): 432 – 442.

Tag, H. and Das, A.K. 2004. Ethnobotanical notes on the hill miri tribe of Arunachal Pradesh. Indian J. Trad. Knowl. 3(1): 80–85.

Tag, H., Das, A.K. and Kalita, P. 2005. Plants used by the hill miri tribe of Arunachal Pradesh in Ethnofisheries. Indian J. Trad. Knowl. 4(1): 57–64.

Tag, H., Jambey, T., Pallabi, K.H., Baikuntha, J.G. and Vijay, V. 2014. Nutritional Potential and Traditional Uses of High Altitude Wild Edible Plants in Eastern Himalayas, India. Int. J. Agr. Biosyst. Sci. Eng. 8(3): 395–400.

Taiz, L. and Zeiger, E. 2010. Augmentation of Dry Matter Production, Photosynthetic Enzymes, Yield Attributes and Quality Parameters of Sunflower through Seed Priming Effect of Gibberellic Acid—A Multifaceted Hormone. Plant Physiology [5th Edition]. Sinauer Associates Inc., Sunderland.

Taktajan. 1996. Indo-Myanmar phytogeographical rims of fareast Himalayan are considered as centre of diversity of flowering. J. Ethnobiol. Ethnomed. 15(4): 43 – 57.

Thothathri, K. and Pal, G.D. 1987. Further contribution to the Ethnobotany of Tribals of Subansiri District of Arunachal Pradesh. J. Econ. Taxon. Bot. 10: 149 – 157.

Tiwari, K.C., Majumdar, R. and Bhattacharjee, S. 1976. Folklore medicine from Assam and Arunachal Pradesh (District Tirap). Pharm. Biol. 17(2): 61–67.

Tiwari, U.L., Kotia, A. and Rawat, G.S. 2009. Medico-ethnobotany of the Monpas in Tawang and West Kameng districts of Arunachal Pradesh, India. Pleione 3(1): 1–8.

Tripathi, A.K., Limasenla and Shankar, R. 2017. Ethno-medicinal plants used by nyishi tribe of Arunachal pradesh, India. World J. Pharm. Pharm. Sci. 6(5): 1246–1253.

Tsering, J. 2016. Ethnobotany and phytochemical analysis of selected Traditional wild food and medicinal plants of the Monpa Community of Arunachal Pradesh (Thesis submitted for PhD). Dept. of Botany, Rajiv Gandhi University. Arunachal Pradesh, India.

Tsering, J., Gogoi, B.J., Hui, P.K., Tam, N. and Tag, H. 2017. Ethnobotanical appraisal on wild edible plants used by the Monpa community of Arunachal Pradesh. Indian J. Trad. Knowl. 16(4): 626–637.

Valsaraj, R., Pushpangadan, P., Smitt, U.W., Adsersen, A., Christensen, S.B., Sittie, A., Nyman, U., Nielsen, C. and Olsen, C.E. 1997. New Anti-HIV-1, Antimalarial, and Antifungal Compounds from Terminalia bellerica. J. Nat. Prod. 60(7): 739–742.

Wangpan, T., Chetry, L.B., Tsering, J., Taka, T. and Tangjang, S. 2016. Anti-Malarial Plants of Jonai, India: an ethnobotanical approach. Not. Sci. Biol. 8(1): 27–32.

Wong, K.C. and Tan, G.L. 1994. Steam volatile constituents of the aerial parts of *Paederia foetida* L. Flavour Frag. J. 9: 25–28.

Xu, X., Lin, Q., He, X., Fu, F. and Chen, G. 2010. Determination of protoberberine alkaloids in medicinal plants based on acidic potassium permanganate chemiluminescence system. Luminescence 25: 403–408.

Yakang, B., Gajurel, P.R., Potsangbam, S. and Bhuyan, L.R. 2013. Account of common and traditional non-timber forest products used by Apatani of Arunachal Pradesh, India. Pleione 7(2): 514–530.

13

Characterization and Purification of Antiurolithiatic Metabolites from Medicinal Plants

Ankit S. Kale,[1] *Anita S. Patil,*[1,*] *Hariprasad Paikrao*[2] and *Surendra R. Patil*[3]

Introduction

Urolithiasis is one of the common disorders in the global population; every 10th person of entire population is suffering with these painful urological disorders (Edvardsson et al. 2013). It is an alarming situation in developing as well as developed countries due to the absence of a permanent remedial solution. It is reported that the percentage of urolithiasis is greater in males than females with a male:female ratio of 3:1 (Stamatelou et al. 2003).

In India, approximately 5–7 million patients suffer from stone disease and nearly 1/1000 of the Indian population needs hospitalization due to kidney stone disease (Pearle et al. 2005). A huge number of patients belong to the age group of almost twenty (Munver and Preminger 2001). The rate of recurrence is about 15% in one year and up to 50% within five years of the initial stone (Spirnak and Resnick 1987).

Urolithiasis is the disorder earlier seen in history back not only to 4000 B.C. in the tombs of Egyptian mummies (4000 B.C.) but also in graves of North American Indians from 1500 to 1000 B.C. (Bahuguna et al. 2009). It is also documented in the early Sanskrit documents during 3000 and 2000 B.C. Urolithiasis is considered as a medical challenge due to its multifactorial etiology and high rate of recurrence. It was reported earlier that super-saturation of urine with salt and minerals such as calcium oxalate (CaOx), struvite (ammonium magnesium phosphate), uric acid and cysteine is actually causing renal calculi formation (Kulaksizoglu et al. 2008, Martin and Nieto 2011, Macneil and Bariol 2011). These calculi are of different sizes and shaped-small 'gravel-like' stones to large stag horn calculi. The crystals stay at their location or move further down the urinary tract, producing symptoms along the way.

In spite of substantial progress in the biological and physical manifestation of urolithiasis, its mechanism is still not clearly understood and there are no satisfactory safe drugs available for the treatment and prevention of urolithiasis. The drugs which are used for prophylactic therapy are primarily aimed to

[1] Lab # 106 Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati (M.S.), India.
[2] Government Institute of Forensic Science, Aurangabad, 431004 (M.S.), India.
[3] College of Horticulture, Dr. Panjabrao Deshmukh Agriculture University, Akola (M.S.), India.
* Corresponding author: anitapatil@sgbau.ac.in

correct the underlying metabolic disorders, but still results are not so convincing. The limited success of chemical drugs in urolithiasis is that multiple factors are involved in its pathogenesis, and the treatment demands multifactorial drug with various biological activities, such as an antispasmodic, antimicrobial, antioxidant, anti-inflammatory, etc. (Khan et al. 2011).

Several medicines like Thiazide diuretics (e.g., Hydrochlorothiazide), alkali, (e.g., Potassium citrate), Allopurinol, Sodium Cellulose Phosphate (SCP), Penicillamine (Cuprimine), Analgesic (Diclofenac sodium), Bisphosphonates, Potassium phosphate, *Oxalobacter formigenes* and other probiotics are used in urolithiasis, which act by decreasing the excretion of the stone forming agent such as oxalates, calcium, phosphates, etc. (Choubey et al. 2010).

The medicinal plants contain multiple chemical constituents, which could offer synergistic or side-effect neutralizing properties that are likely to offer a more effective and safer remedy. Therefore, there is a need to look for an alternative therapy, especially herbal remedies, for the management and treatment of urolithiasis (Butterweck and Khan 2009).

Now-a-days, herbal medicine has gained much popularity being more efficient, easily available, having a low cost, with fewer side effects and the reducing recurrence rate of stone formation, hence search for antilithiatic drug from natural sources has assumed greater importance. In Ayurveda, many plants having the property of disintegrating and dissolving the stone are collectively referred to as "Pashanbheda" (Agarwal and Varma 2014). Plant extracts contain phytochemicals that prevent stone formation by inhibiting synthesis and agglomeration of crystals (Bhattacharjee et al. 2012).

Recently, significant progress has been made in identifying and quantifying physicochemical processes responsible for urinary stone formation. It is evident that super-saturation of urine with calcium oxalate is essential for urinary calcium oxalate crystallization (Kulaksizoglu et al. 2008). The best ways to prevent and treat urolithiasis are to control the process of crystallization events and most important is controlling the initial step, i.e., nucleation step. This is exceptionally achieved using herbal extracts since they have been widely used in folk medicine to treat kidney stones. The idea to use herbal extracts in the first step is that if nucleation itself is stopped or controlled, the next steps which lead to formation, aggregation and retention of crystals do not occur at all. In present chapter, we propose methods based on confirmation of antilithiatic plant bioactive metabolites including their purification and preliminary characterization.

Urolithiasis

Kidney stone remains one of the major painful problems of a human population worldwide. Formation of stones in the urinary system, i.e., in the kidney, ureter and urinary bladder or in the urethra is Urolithiasis (Chandrasoma and Taylor 1998). There are several factors responsible for formation of stones such as climate, geographical distribution, food habits, changing lifestyle, genetic factors, etc. (Anderson 1979). Urolithiasis, kidney stones, nephritic stones, and renal calculi are interchangeably used to refer to the accretion of hard, solid, non-metallic minerals anywhere in the urinary system, including the kidneys, urethra and the bladder. There are five different types of stones reported, including calcium oxalate stones, calcium phosphate stone, magnesium ammonium phosphate, cysteine and uric acid stone (Free and Free 1975), and many are yet to be identified (Table 13.1). Common stones among all are types of renal stones CaOx, which are of two types, i.e., monohydrate type (in the form of dump bell or oval) and dihydrate type (in the form of the double pyramid) (Kannabiran and Selva 1997). Hydrated forms of CaOx are reported as: whewellite (monohydrate, known from some coal beds), weddellite (dihydrate) and a very rare trihydrate called caoxite. These crystals look like a six sided prism and often look like a pointed picket from a wooden fence.

Calcium oxalate Stones

Calcium oxalate Stone (CaOx) is the most common type of stone found in different renal type of stones. It was found that approximate 80% of stone patient cases in US were CaOx type; these CaOx may be alone or may be in combination with calcium phosphate in the form of apatite or brushite (Coe et al. 2003). Hyperoxaluria, hyperparathyroidism and renal tubular acidosis are some factors that promote the

Table 13.1 Types of kidney stones found in renal disorder.

Name of stone	Approximate incidence	Constituents
CaOx	70% of all stones	Calcium, oxalate
Calcium phosphate	10% of all stones	Calcium, phosphate
Uric acid	5–10% of all stones	Uric acid
Struvite	10% of all stones	Calcium, ammonia, phosphate
Cystine	Less than 1% of all stones	Cystine
Medication-induced stones	Less than 1% of all stones	Composition depends on medication or herbal product (e.g., include indinavir, ephedrine, guaifenesin, silica)

precipitation of CaOx and calcium phosphate stones (Hoppe and Langman 2003). Hyperoxaluria is seen in patients who eat more oxalate containing food like vegetables and nuts. Many drugs are available in the market for treatment of urolithiasis with significant results, which include allopurinol, tri-sodium citrate, Cystone, thiazide diuretics, etc. Some surgical procedures are also used in routine practices to remove stones, which include Ureteroscopy; Percutaneous nephrolithotomy (PCNL), Extracorporeal Shock Wave Lithotripsy (ESWL) and open surgery are applied to remove kidney stones. These therapies are very costly, painful, and recur along with a side effect (Fig. 13.1), which strongly calls for new treatment options. The lowered side effects associated with herbal medicines have reignited interest in phytomedicine (Fig. 13.2). The Ayurveda system of medicine, which is widely followed in India, provides a solid foundation to search new herbal formulations having the ability to act on stones. All such factors make this area interesting for the researcher's aim of hunting new possible solutions for human welfare (Aeckart and Schroder 1989).

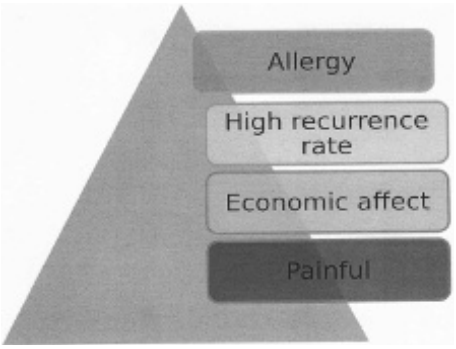


Fig. 13.1 Some effects of Urolithiasis.

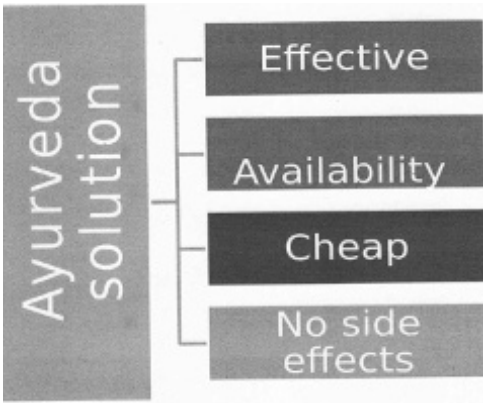


Fig. 13.2 Benefits of Ayurvedic treatment.

Medicinal plants for treatment of urolithiasis

Medicinal plants are as old as mankind itself and connecting man and his search for drugs in nature from the far past, as found in written documents, preserved monuments, and even original plant medicines of ancient times. People living in the interiors and inaccessible remote rural areas have excellent knowledge about medicinal utility of the local flora, and they have strong faith in their own folklore preparations or crude formulations. The knowledge of medicinal plants is applied against illnesses due to which man learned to pursue drugs in different plant parts. Based on scientific validation such plants have been included in modern pharmacotherapy. Studies related to the usage of medicinal plants as well as awareness have been increased which has enhanced the ability of medical professionals to respond to the challenges.

Modern science has taken advance steps in the field of medicine, but use for conventional medicine is in the primary form available to the people in developing countries, including India. It is observed that about 80% of the population of developing countries still believes in traditional medicine for basic health issues (Ekor 2013). India has a rich heritage of traditional medicine, and the traditional health care system has been flourishing in many countries. Still today such medicines are not incorporated in most national health systems, and the potential of services provided by the traditional practitioners (Mukerjee 2002). During the last decade, the use of herbal medicine has increased worldwide, which has provided an excellent opportunity in India to look for therapeutic lead compounds from an ancient system of therapy, i.e., Ayurveda. Over 50% of all modern drugs are of natural product origin, which play an important role in drug development programs of the pharmaceutical industry (Baker et al. 1995).

Recent studies are trying to find out the exact mechanism and effectiveness of such traditional drugs against different stages of urolithiasis, such as: the diuretic action increases the quantity of fluid going pass through the kidneys and as a result flush out the deposits (Gohel and Wong 2006). It has been reported that crystal inhibitors in plants are supposed to decrease crystal nucleation, aggregation and growth (Patil et al. 2017, Kale et al. 2017). Furthermore, they also inhibit crystallization by their adsorption to the crystal surface which makes them unable for renal tubular attachment (crystallization inhibition activity). The formation of stones in the urinary system leads to lipid peroxidation producing Reactive Oxygen Species (ROS) followed by renal cell injury and inflammation. It also includes loss of membrane integrity, promotes fibrosis and collagen formation, and facilitates CaOx retention and subsequent stone formation (Khan 2013). Plant drugs are in the demand worldwide because of their higher potential to treat diseases, safety margin and less cost (Banso and Ngbde 2006). The increasing interest in traditional phytomedicine may lead to the discovery of novel therapeutic agents. Although herbs are supposed to be safe, many unsafe and fatal side effects have recently been reported due to dose and unprescribed application (Ikegami et al. 2003, Izzo 2004). Remedial plants possess various medicinal properties, which includes antiviral, antilithiatic, anti-inflammatory, antimicrobial, antioxidant, cytotoxic, hepatoprotective, antidiabetic, insecticidal, antilarval and many more (Marino and Bersani 1999, Nickavar et al. 2005, Nolkemper et al. 2006, Szczepanik et al. 2012, Amiri 2012, Chang et al. 2017).

Antiurolithiatic drugs available from the medicinal plants are the easiest and well known alternative sources, which are cost-effective and with the least side effects. Such herbal drugs are in great demand in developing and developed countries because of their potential to treat diseases, safety margin and less cost. A large number of Indian medicinal plants have been used in the treatment of urolithiasis, which is reported to be effective with no side effects. Their extracts were prepared in different solvent by different methods; different assays were used to analyze their potential, as shown in Table 13.2.

Extraction and purification of antilithiatic compounds

Medicinal plants are the important natural resource for novel therapeutic compounds. Although many approaches are available for the discovery of pharmaceutical constituents, the plant remains major reservoirs of new structural types. Plants are capable of synthesizing a diverse array of secondary metabolites, which include tannins, terpenoids, coumarin, alkaloids and flavonoids (Perez and Anesini 1994). These metabolites may be produced constitutively or in response to pathogen or herbivore attack or stress (phytoalexins) (Wittstock and Gershenzon 2002). Out of the several hundred thousand plant species over the globe, only a small proportion has been investigated both phytochemically and pharmacologically. The crucial

Table 13.2 A list of medicinal plants with antilithiatic property.

S. No.	Botanical name	Common names	Plant part	Solvent extract	*In vitro*/*in vivo* assay	Reference
1.	*Boerhaavia diffusa* L.	Gadha-khand	Whole plant	Aqueous	*In vitro*	(Pareta et al. 2010)
2.	*Acalypha indica* L.	Indian nettle	Whole plant	Ethanol	*In vivo*	(Sathyaa et al. 2011)
3.	*Achyranthes indica* L.	Chirchira	Roots	Hydro alcoholic	*In vitro*	(Patera et al. 2010)
4.	*Achyranthes aspera* L.	Putkhanda, Prickly chaff flower	Roots	Aqueous	*In vitro*	(Aggarwal et al. 2010)
5.	*Achyranthesindica* L.	Chirchira	Roots	Hydroalcoholic	*In vitro*	(Pareta et al. 2011)
6.	*Aerva lanata* L.	Sirupoolai, Chaya	Whole plant	Aqueous	*In vivo*	(Soundararajan et al. 2006)
7.	*Ageratum conzoides* L.	Chick weed	Whole plant	Hydro alcoholic	*In vivo*	(Khan et al. 2011)
8.	*Alismatis rhizome* (Sam.) Juzepcz.	Takusha	Whole plant	Aqueous	*In vitro*	(Suzuki et al. 1999)
9.	*Argemone mexicana* L.	Datturigida	Leaves	Aqueous	*In vitro*	(Chilivery et al. 2016)
10.	*Asparagus racemosus* W.	Shatavari	Roots	Ethanol	*In vivo*	(Jagannath et al. 2012)
11.	*Bergenia ligulata* W.	Paashaanbhed	Rhizomes	Methanol, Aqueous	*In vitro*	(Bashir and Gilani 2009)
12.	*Beta vulgaris* L.	Ullamgadda	Roots	Aqueous	*In vitro*	(Saranya and Geetha 2014)
13.	*Boerhaaviadiffusa* L.	Hogweed	Whole plant	Aqueous	*In vitro*	(Pareta et al. 2010)
14.	*Bryophyllum pinnatum* (Lam.) Oken	Patharchatta	Leaves	Aqueous	*In vitro*	(Patil et al. 2015)
15.	*Celosia argentea* L.	Silver Cockscomb	Seed	Ethanol	*In vivo*	(Joshi et al. 2012)
16.	*Ceropegia bulbosa* Roxb.	Hedulo	Root	Ethanol, Aqueous	*In vitro*	(Monika et al. 2012)
17.	*Citrus limon* (L.) Osbeck	Lemon	Fruit	Aqueous	*In vitro*	(Kulaksizoglu et al. 2008)
18.	*Citrus medica* L.	Bara nimbu	Fruit	Aqueous	*In vitro*	(Kalpeshsinh et al. 2012)
19.	*Coleus aromaticus* Benth.	Indian borage	Leaves	Hydro alcoholic	*In vivo*	(Venkatesh et al. 2010)
20.	*Convulvus arvensis* L.	Bindweed	Leaf, flower	Aqueous	*In vitro*	(Rajeshwari et al. 2013)
21.	*Costus arabicus* L.	Crepe Ginger	Aerial part	Aqueous	*In vitro*	(De Cagoin et al. 2015)
22.	*Cynodon dactylon*	Bermuda grass	Root	Hydro alcoholic	*In vivo*	(Ashok Kumar et al. 2013)
23.	*Glochidion veluntinum*	Tshangla	Leaf	Methanol	*In vivo*	(Vijaya et al. 2013)
24.	*Hygrospila spinosa*	Ikshura	Leaf	Aqueous	*In vivo*	(Satish et al. 2010)

Table 13.2 contd. ...

...Table 13.2 contd.

S. No.	Botanical name	Common names	Plant part	Solvent extract	*In vitro*/*in vivo* assay	Reference
25.	*Hyptis suaveolens*	Bilatti	Aerial part	Ethanol	*In vitro*	(Agarwal and Varma 2012)
26.	*Kalanchoe pinnata*	Stone breaker	Leaf	Aqueous	*In vitro*	(Pathak and Hendre 2015)
27.	*Lantana camara* L.	Spanish flag	Leaves	Aqueous	*In vitro*	(Reddy 2013)
28.	*Launaea procumbens*	Pathari	Leaf	Methanol	*In vivo*	(Makasan et al. 2014)
29.	*Lawsonia* L. inermis	Henna	Leaves	Aqueous	*In vivo*	(Kore et al. 2011)
30.	*Melia azadirachta*	Chinaberry tree	Aerial part	Aqueous	*In vivo*	(Tina et al. 2006)
31.	*Melia dubai* C.	Malabar Neem	Leaves	Aqueous	*In vitro*	(Venilla and Mariyal 2015)
32.	*Mimusops elengi* L.	Spanish cherry, Bullet wood	Bark	Ethanol, Aqueous	*In vivo*	(Ashok et al. 2010)
33.	*Moringa oleifera*	Drum stick tree	Pods, bark	Aqueous	*In vivo*	(Fahad et al. 2010)
34.	*Musa paradisica* L.	Banana plantain	Stem	Aqueous	*In vivo*	(Thirumala et al. 2013)
35.	*Ocimum gratissimum*	African basil	Arial part	Ethanol	*In vitro*	(Agarwal and Varma 2014)
36.	*Orthosiphon stamineus*	MisaiKucing	Leaves	Ethanol	*In vivo*	(Ramesh et al. 2014)
37.	*Pergularia daemia* Forssk	Dustapuchettu	Whole plant	Hydroalcohlic	*In vivo*	(Vyas et al. 2011)
38.	*Phyllanthus niruri* L.	Stone breaker	Leaves	Aqueous	*In vitro*	(Patil et al. 2015)
39.	*Pinus eldarica* M.	Goldwater pine	Fruits	Aqueous	*In vivo*	(Hosseinzadeh et al. 2010)
40.	*Portulaca oleracea*	Green Purslane	Leaves	Ethanol	*In vivo*	(Kishore et al. 2013)
41.	*Rotula aquatic* L.	Pashannabedha	Roots	Chloroform, Aqueous	*In vitro*	(Gilhotra et al. 2011
42.	*Solanum virginianum* L.	Bari kateli	Whole plant	Ethanol	*In vivo*	(Chinnala et al. 2013)
43.	*Tamarix gallica* L.	Aabda	Leaves	Diethyl ether	*In vitro*	(Bensatal and Quahrani 2008)
44.	*Tecoma stans*	Yellow bells	Leaves	Aqueous	*In vivo*	(Kameshwaran et al. 2013)
45.	*Tribulus terrestris* L.	Chhotagokhru	Fruits	Aqueous	*In vitro*	(Patil et al. 2015)
46.	*Withenia somnifera*	Winter cherry	Fruits	Methanol	*In vivo*	(Patel and Mandal 2014)
47.	*Zea mays* L.	Makki, Makka	Styles	Aqueous	*In vitro*	(Rathod et al. 2013)
48.	*Zingiber officinale* R.	Ginger, Sunthi	Rhizomes	Ethanol	*In vivo*	(Lakshmi and Divya 2014)

factor in investigation of bioactive plant metabolites is the selection of plant material or their parts for the optimum amount of phytoconstituents.

The robust bioassays and targeted isolation of bioactive compounds are need for current pharmacological research. The major problem in ethnopharmacology research is to isolate and characterize the molecules from extracts and their purification. The extensive fractionation of extracts may lead to reduction or loss of biological activity due to break down or loss of additive or synergistic effects between analogue constituents. Some modern extraction techniques are also available, including solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, which possess advantages over the traditional methods.

All these methods save solvents, reduces the time, sample degradation, elimination of additional sample. These methods reduced concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and kinetics of extraction. This automation also favours their usage for the extraction of plant's materials (Huie 2002). Extraction is an important step in the analysis of medicinal plants; it gives the opportunity to extract the desired chemical compounds for separation and characterization.

The basic operation include steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to ensure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples.

Purification of antilithiatic metabolites

Plants provide an excellent opportunity for new drug discovery because of the unmatched availability of chemical diversity (Cos et al. 2006). It is clear that plant extracts usually occur as a combination with various types of bioactive compounds or phytochemicals with different polarities. However, their isolation, purification, separation still remains a challenging task in the process of identification and characterization of bioactive compounds. Some common techniques used for isolation, and purification included Thin Layer Chromatography, column chromatography, flash chromatography; Sephadex chromatography and HPLC are useful in obtaining the pure compounds. The pure compounds can be used for determination of structure and biological activity. TLC bioautography and column chromatography is becoming an important tool in the isolation of bioactive metabolites of plant crude extract.

TLC bioautography

A novel Thin-Layer Chromatography (TLC)-direct bioautography method was also proposed to detect the antilithiatic metabolites separates on TLC plate (Patil et al. 2017). The CaOx inhibitors or constituents of plant extracts are separated on TLC and comes in contact with agar; these metabolites diffuse in gel beads of agar to form a zone of inhibition. The clear zones formed against CaOx crystals in the gel confirms the antilithiatic potential. Such separated bioactive band can be scrapped from TLC and identified by the spectroscopic and chromatographic technique, as shown in Fig. 13.3 (Patil et al. 2017, Indian Patent no. 494/MUM/2013, Method for identification of metabolites possessing calcium oxalate stone inhibitory properties in plant extract using TLC bioassay).

Column chromatography

Column chromatography is one of the powerful tools used in separation of pure compounds into a crude drug. This drug can be purified by using a gradient of solvents of different polarity in different times. The fraction of different polarity separates based upon their solubility in the solvent. These fractions can be collected in distinct tubes, and vacuum dried. The entire fraction should be screened by antiurolithiatic assays such as slide gel assay, agar gel assay or crystallization assay; the most potent antilithiatic fraction compounds can be identified by spectroscopic and chromatographic technique such as HR-LCMS.

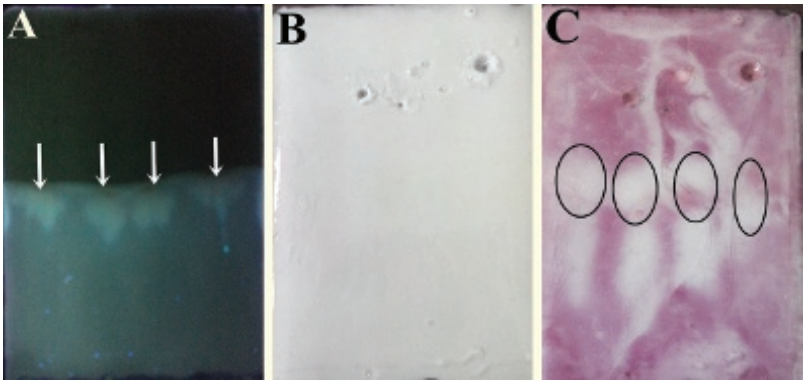


Fig. 13.3 TLC bioautography for detection of CaOx inhibition by tri-sodium citrate. (A) TLC plate under UV light 365 nm (arrows showing bands of tri-sodium citrate). (B) TLC plate after over layered with calcium containing agar gel. (C) TLC plate after ammonium oxalate treatment and staining with Alizarin red S for detection of CaOx inhibition (Circles showing region of inhibition).

Secondary metabolites reported in various plants for antilithiatic purpose

There are several phytochemicals which could be accountable for the antilithiatic effect. According to Arafat et al. (2008), flavonoids and triterpenes have a key role in preventing urolithiasis. It is also believed that saponins and tannins act as antiurolithiatic phytoconstituents (Doddola et al. 2008). Soundararajan et al. (2006), reported the dissolution of CaOx crystals due to effect of flavonoids (Kaemepferol-3-rhamnoside and kaempferol-3-rhamnogalactoside), triterpenes (betulin) and tannins. It is also reported that saponin rich fractions of other plants like, *Herniaria hirsuta* act as a great inhibitor of calcium stone formation under *in vitro* and *in vivo* model studies (Fouada et al. 2006). Lupeol and betulin (triterpenes) have been found to be efficient in reducing the risk of stone formation in animals by way of preventing crystal-induced tissue damage and dilution of urinary stone-forming constituents (Malini et al. 2000). Patil et al. (2017) reported the presence of antiurolithiatic plant metabolites including tuberonic acid, Methyl-8-(2-(2-formyl-vinyl)-3-hydroxy-5-oxy-cyclopentyl) octanoate, 9-hexadecen-1-ol, 1-hexadecanoyl-sn-glycero-3-phosphocholine.

Screening methods

A large number of medicinal plants are reported with an antiurolithiatic activity. So it would an area of interest to find the most potent antilithiatic potential of plants. There are some *in vitro* and *in vivo* rat model methods reported to find and validate the activity. The studies are routinely employed for screening medicinal plants with respect to their antilithiatic property in inhibiting/assessing the nucleation, aggregation and growth inhibition of urinary stone constituents. The chemical analysis of kidney stones shows that most of the urinary stones predominantly consists of CaOx and calcium phosphate. Hence, most of the studies to assay or screen the antiurolithiatic property of medicinal plants were done by initial screening of the crude drugs by standardized *in vitro* gel method of crystallization (Henisch et al. 1970, 1988) or by *in vitro* method of Baumann and Wacker (1980) or by *in vivo* rat model experiments . The effect of *Crataeva nuruala* bark decoction on CaOx urolithiasis induced by 3% glycolic acid has been studied in rats.

Many *in vitro* bioassays were performed including slide gel assay (Schneider et al. 1983), agar gel overlay assay (Patil et al. 2014), dot blot assay (Kale et al. 2017), urine assay (Patil et al. 2015), and microscopic crystallization assay (Patil et al. 2017) as shown in Fig. 13.4.

Slide gel assay

Modified Schneider slide gel method was used for the *in vitro* study of potential of antiurolithiatic medicinal plants. The potential can be calculated by measuring the inhibitory area formed by the plant drug and the negative control using the formula as below (Schneider et al. 1983).

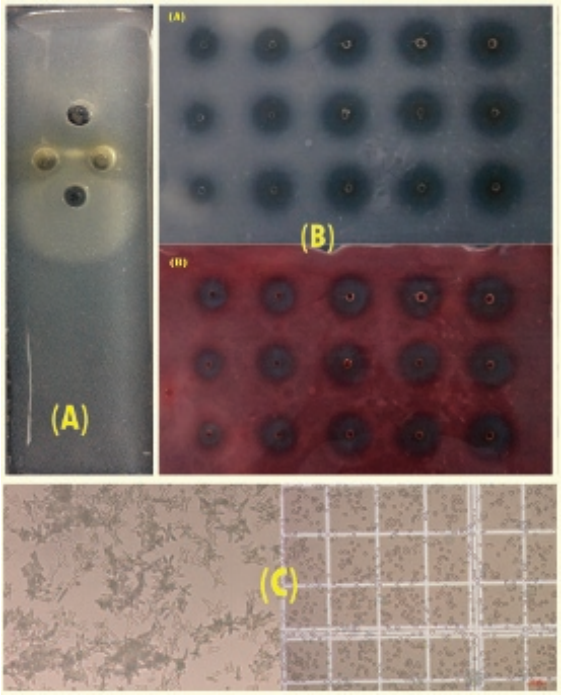


Fig. 13.4 Some *in vitro* assays used for urolithiasis (A) slide gel assay (B) agar gel overlay assay (C) microscopic crystallization assay.

Inhibitory indexes can be calculated by:

$$I = 1–As/Ac \times 100$$

Where, As = area of calcium oxalate crystals in presence of sample tested and
Ac = area of calcium oxalate crystals formed for the corresponding blank.

Agar gel overlay assay

Agar gel overlay method is one of the most significant methods for qualitative and quantitative potential of compounds and any plant extracts. The potential of the plant drug can be measured by calculating the inhibitory area and percentage area of zones of inhibition using the formula as suggested by Patil et al. (2014) in the Indian Patent No. 1501/MUM/2014; the method for *in vitro* qualitative and quantitative detection of calcium oxalate inhibitory activity.

Percent Inhibition can be calculated by:

$$PI = 100–(1–(As/Ac)) \times 100$$

Where

As = Area of diffused sample; Ac = Area of control region

Microscopic Crystallization Assay

Microscopic Crystallization Assay is one of potent methods, which screens out the plants antiurolithiatic potential on the basis of change in crystal number, size and shape. The method shows visible change in number, size and shape under the microscope and gives qualitative and quantitative data. Many *in vivo* models have been developed to understand the mechanisms involved during the formation of urinary stones and to ascertain the effects of various therapeutic agents on development and progression of the disease.

CaOx kidney stones in both humans and mildly hyperoxaluria rats are located on renal papillary surfaces and consist of an organic matrix and crystals of CaOx. The rat is the most frequently used animal to induce CaOx deposition into kidneys and mimic the etiology of the formation of stones in humans. Stones formed in kidneys of humans, and rats are identical and exhibit similarities between human and rats CaOx kidney stones in many aspects.

Hyperoxaluria, defined as excessive urinary oxalate, is the main risk factor for human idiopathic CaOx stone formation and induction of hyperoxaluria is essential for the development of CaOx urolithiasis in rats (Khan 1995). CaOx stone is the most prevalent component in urolithiasis, and many experimental models have been used to demonstrate its formation in the animal kidney (Khan et al. 1982, Khan 1995). Ethylene Glycol (EG), which is a precursor to oxalate formation, has generally been used in combination with ammonium chloride (NH_4Cl) or vitamin D_3 in an attempt to form CaOx crystals in urine and CaOx deposits in the kidney of rats (Yamaguchi et al. 2005). Urinary excretion of oxalate will be increased during the chronic administration of ethylene glycol (EG) as a 0.75% aqueous solution in drinking water to male Sprague-Dawley rats.

Future perspectives

In developing countries, most of the population uses medicinal plants as their home-based treatment. Herb based antiurolithiatic drugs are available in medical stores; they are cost effective, easily available and without side effects. The potential of such drugs is widely accepted by the people. Different companies are selling these drugs in the market with variable content, different name and in a different form; some drugs are Cystone of Himalaya, Gokru Kada of Baidyanath and many more. Recent technology with traditional medicine has opened the new channels in the field of improving health; such treatment is especially useful for people, who cannot afford costly western medicines. These researches also generate the opportunity for the Indian farmers to produce medicinal plants having economic importance. This clearly opens door for policy makers, researchers, industry and farmers to manage important medicinal plants.

Conclusion

The growing resurgence and revival of interest in indigenous systems of medicine and traditional herbal remedies, especially for their use for urolithiasis are regarded as quite safe. The best way to prevent and treat urolithiasis is to control the process of crystallization events from initial step, i.e., nucleation. Many herbs themselves possess inhibitory activity against crystallization, and their antioxidant activity helps in preventing the urolithiasis renal cell damage.

In the present chapter we have described quantitative/qualitative antiurolithiatic potential of medicinal plants by using *in vitro* assays viz. slide gel assay, agar gel overlay assay and microscopic crystallization assay. The proposed *in vitro* assays conclude that plants possess tremendous potential to stop the growth of crystals; and shows special mechanism of reducing the size and number of the crystal which help in removing them from the urinary system. Agar Gel Overlay Assay is supposed to be a more efficient method compared to conventional methods, as it saves time and interprets very clear measurable results.

As the previously reported the slide gel method is sensitive, but time consuming, and also calculation is very difficult and with chances of human error, which can be overcome by the proposed methods. Furthermore, the plant extracts prepared by maceration method in water confirmed as the most potent method for the crude drug extraction in terms of antilithiatic potential. Although the use of herbal medicines are popular and promising, it is essential to carry out further research to understand the pathophysiology of disease, and mechanism of action in order to develop an efficient and safe litholytic agent.

Acknowledgment

The authors would like to thank Rajiv Gandhi Science and Technology Commission, Mumbai, India for research grant (RGSTC/File-2012/DPP-94/CR-12) under the major research project sanctioned to Prof. Anita Patil and also to Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati (M.S.) India for providing the research facilities.

References

Aeckart, K.S.J. and Schroder, F.H. 1989. Effect of extra corporeal shock wave lithotripsy (ESWL) on renal tissue. Urol. Res. 17: 3–7.

Agarwal, K. and Varma, R. 2012. Inhibition of calcium oxalate crystallization *in vitro* by various extracts of *Hyptis suaveolens* (L.) PIOT. Int. Res. J. Pharm. 3(3): 261–264.

Agarwal, K. and Varma, R. 2014. *Ocimum gratissimum* L.: A medicinal plant with promising antiurolithiatic activity. Int. J. Pharm. Sci. Drug. Res. 6(1): 78–81.

Aggarwal, A., Tandon, S., Singla, S.K. and Tandon, C. 2010. Diminution of oxalate induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization *in vitro* by aqueous extract of *Tribulus terrestris*. Int. Braz. J. Urol. 36(4): 480–489.

Amiri, H. 2012. Essential oils composition and antioxidant properties of three thymus species. Evid.-Based Compl. Alt.: 728065. doi:10.1155/2012/728065.

Anderson, C.K. 1979. The anatomical aetiology of renal lithiasis. pp. 40–68. *In*: Wickham, J.E.A. (ed.). Urinary Calculous disease. Edinburgh: Churchill Livingstone.

Arafat, O.M., Tham, S.Y., Sadikun, A., Zhari, L, Haughton, P.J. and Asmawi, M.Z. 2008. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats. J. Ethnopharmacol. 118(3): 354–360.

Ashok Kumar, K., Selvaraj, K. and Muthukrishnan, S.D. 2013. Cynodondactylon (L.) Pers.: An updated review of its phytochemistry and pharmacology. J. Med. Plants Res. 7(48): 3477–83.

Ashok, P., Koti, B.C. and Vishwanathswami, A.H.M. 2010. Antiurolithiatic and antioxidant activity of *Mimuspos elengi* on ethylene glycol-induced urolithiasis in rats. Ind. J. Pharmacol. 42(6): 380–386.

Bahuguna, Y.M., Rawat, M.S.M., Juya, V. and Gnanarajan, G. 2009. Antilithiatic effect of grains of *Eleusine coracana*. Saudi Pharm. J. 17(2): 182–188.

Baker, J.T., Borris, R.P., Carte, B., Cordell, G.A. and Soejarto, D.D. 1995. Natural product drug discovery and development: New perspective on international collaboration. J. Nat. Prod. 58: 1325–57.

Banso, A. and Ngbde, J.E. 2006. Phytochemical screening and *in vitro* antifungal properties of *Fagara zanthoxyloides*. J. Food Agric. Environ. 4(3 and 4): 8–9.

Bashir, S. and Gilani, A.H. 2009. Antiurolithiatic effect of *Bergenia ligulata* rhizome: an explanation of the underlying mechanisms. J. Ethnopharmacol. 122: 106–116.

Baumann, J.M. and Wacker, M. 1980. The direct measurement of inhibitory capacity to crystal growth of calcium oxalate in undiluted urine and in other inhibitor containing solutions. Urol. Res. 8(3): 171–5.

Bensatal, A. and Ouahrani, M.R. 2008. Inhibition of crystallization of calcium oxalate by the extraction of *Tamarix gallica* L. Urol. Res. 36: 283–287.

Bhattacharjee, A., Shashidhara, S.C. and Aswathanarayana. 2012. Phytochemical and ethno-pharmacological profile of *Cratraeva nurvala* Buch-Hum (Varuna): A review Asian Pac. J. Trop. Biomed. 45: 1162.

Butterweck, V. and Khan, S.R. 2009. Herbal medicines in the management of urolithiasis: alternative or complementary? Planta Med. 75: 1095–1103.

Chandrasoma, P. and Taylor, C.R. 1998. Concise Pathology. 3rd Edition. USA: Appleton & Lange; The ureter, urinary bladder & urethra, pp. 738–739.

Chang, N., Luo, Z., Li, D. and Huiying Song. 2017. Indigenous Uses and Pharmacological Activity of Traditional Medicinal Plants in Mount Taibai, China. Evid.-Based Compl. Alt. 1–11.

Chilivery, R.K., Alagar, S. and Darsini, T.P. 2016. *In vitro* Anti-Urolithiasis Potentials of *Argemone mexicana* L. Leaves. Curr. Clin. Pharmacol. 11(4): 286–290.

Chinnala, K.M., Shanigarm, S. and Elsani, M.M. 2013. Antiurolithiatic activity of the plant extracts of *Solanum virginianum* on ethylene glycol induced urolithiasis in rats. Int. J. Pharm. Biol. Sci. 3(4): 328–334.

Choubey, A., Parasar, A., Choubey, A., Iyer, D., Pawar, R.S. and Patil, U.K. 2010. Potential of medicinal plants in kidney, gall and urinary Stones. Int. J. Drug Dev. Res. 2(2): 431–447.

Coe, F.L., Evan, A. and Worcester, E. 2003. Kidney stone disease. J. Clin. Invest. 115 (10): 2598–2608.

Cos, P., Vlietinck, A.J., Berghe, D.V. and Maes, L. 2006. Anti-infective potential of natural products: How to develop a stronger *in vitro* 'proof-of-concept'. J. Ethnopharmacol. 106: 290–302.

De Cagoin, M.R., Linnes, M.P., Lee, H.J., Krambeck, A.E., De Mendonca Uchoa, J.C., Kim, S.H. and Lieske, J.C. 2015. Aqueous extract of *Costus arabicus* inhibits calcium oxalate crystals and adhesion to renal epithelial cells. Urolithiasis. 43: 119–124.

Doddola, S., Pasupulati, H., Koganti, B., Koganti, V.S.R. and Prasad, G. 2008. Evaluation of *Sesbania grandiflora* for antiurolithiatic and antioxidant properties. J. Nat. Med. 62(3): 300–307.

Edvardsson, V.O., Goldfarb, D.S., Lieske, J.C., Beara-Lasic, L., Anglani, F., Milliner, D.S. and Palsson, R. 2013. Hereditary causes of kidney stones and chronic kidney disease. Pedriat. Nephrol. 28(10): 1923–1942.

Ekor, M. 2013. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 4(177): 1–10.

Fahad, J., Vijayalakshmi, M.C., Kumar Satish, Sanjeeva, Kodancha, G.P., Adarsh, B., Udupa, A.L. and Rathnakar, U.P. 2010. Antiurolithiatic activity of aqueous extract of bark of *Moringa oleifera* (Lam.) in rats. Health. 2(4): 352–355.

Fouda, A., Yamina, S., Nait, M.A., Mohammed, B. and Abdlekrim, A. 2006. *In vitro* and *in vivo* antilithiatic effect of saponin rich fraction isolated from *Herniaria hirsute*. J. Bras. Nefrol. 28, pp. 199–203.

Free, A.H. and Free, H.M. 1975. Renal Lithiasis. In Urinalysis in Clinical Laboratory Practice, CRC Press, pp. 229–243.
GilhotraUmesh, K.R. and Christina, A.J.M. 2011. Effect of *Rotula aquatica* Lour. on ethylene glycol induced urolithiasis in rats. Int. J. Drug Deliv. Res. 3: 273–280.
Gohel, M.D. and Wong, S.P. 2006. Chinese herbal medicines and their efficacy in treating renal stones. Urol. Res. 34(6): 365–372.
Henisch, H.K. 1970. Crystal Growth in Gels, The Pennsylvania State University Press, USA, pp. 93.
Henisch, H.K. 1988. Crystals in Gels and Liesegang Rings, Cambridge University Press, Cambridge. pp. 15–22.
Hoppe, B. and Langman, C.B. 2003. A United States survey on diagnosis, treatment, and outcome of primary hyperoxaluria. Pediatr. Nephrol. 18(10): 986–91.
Hosseinzadeh, H., Khooei, A., Khashayarmanesh, Z. and Shariaty, V.M. 2010. Antiurolithiatic activity of *Pinus eldarica* Medw. fruits aqueous extract in rats. Urol. J. 7(4): 232–237.
Huie, C.W. 2002. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. Anal. Bioanal. Chem. 373: 23–30.
Ikegami, F., Fujii, Y., Ishihara, K. and Satoh, T. 2003. Toxicological aspects of Kampo medicines in Clinical Use. Chem. Biol. Interac. 145: 235–250.
Izzo, A.A. 2004. Drug interactions with St. John's Wort (*Hypericum perforatum*): a review of the clinical evidence. Int. J. Clin. Pharmacol. Ther. 42: 139–148.
Jagannath, N., Chikkannasetty, S.S., Govindadas, D. and Devasankaraiah, G. 2012. Study of antiurolithiatic activity of *Asparagus racemosus* on albino rats. Indian J. Pharmacol. 44(5): 576–9.
Joshi, P.C., Patil, S.A. and Sambrekar, S.N. 2012. The antiurolithiatic activity of ethanolic extract of *Celosia argentea* (seeds) in rats. Universal J. Pharm. 1(1): 52–60.
Kale, A.S., Patil A.S. and Paikrao, H.M. 2017. A modified TLC bioautographic technique for the detection of antilithiatic potential of therapeutic plants from Indigenous Ayurvedic System. Pharm. Methods. 8(1): 209–212.
Kalpeshsinh, C.S., Kumar, F.N., Kirti, P.V., Kalpana, P.G. and Tejal, G.R. 2012. J. Drug Deliv. Ther. 2(4): 109.
Kameshwaran, S., Thenmozhi, S., Vasuki, K., Dhanalakshmi, M. and Dhanapal, C. 2013. Antiurolithiatic activity of aqueous and methanolic extracts of *Tecoma stans*. Flowers in rats. Int. J. Pharm. Biol. Arch. 4(3): 446–450.
Kannabiran, K. and Selva, M.R. 1997. Induction of renal nukleus oxalate binding activity in experimental hyperoxaluric rats. Nephron. 75: 219–223.
Khan, A., Bashir, S., Khan, S.R. and Gilani, A.H. 2011. Antiurolithic activity of *Origanum vulgare* is mediated through multiple pathways. BMC Complem. Altern. M. 11: 96.
Khan, M.A. and Pradhan, D. 2011. Antiurolithic activity of *Ageratum conzoides* extract in rats. Pharmacol. Online. 3: 953–958.
Khan, S.R. 1995. Calcium-oxalate crystal interaction with renal tubular epithelium, mechanism of crystal adhesion and its impact on stone development. Urol. Res. 23: 71–79.
Khan, S.R., Finlayson, B. and Hackett, R.L. 1982. Experimental calcium-oxalate nephrolithiasis in the rat - role of the renal papilla. Am. J. Pathol. 107: 59–69.
Khan, S.R. 2013. Reactive oxygen species as the molecular modulators of CaOx kidney stone formation: evidence from clinical and experimental investigations. J. Urology 189(3): 803–811.
Kishore, D.V., Moosavi, F. and Varma R.K. 2013. Effect of ethanolic extract of *Portulaca oleracea* Linn. on ethylene glycol and ammonium chloride induced urolithiasis. Int. J. Pharm. Pharm. Sci. 5(2): 134–140.
Kore, K.J., Shete, R.V., Jadhav, P.J. and Kabra, M.P. 2011. Antiurolithiatic effects of hydroalcoholic extract of *Lawsonia inermis* L. leaves. Int. J. Uni. Pharm. Life Sci. 1(2).
Kulaksızoglu, S., Sofikerim, M. and Cevik, C. 2008. *In vitro* effect of lemon and orange juices on calcium oxalate crystallization. Int. Urol. Nephrol. 40: 589–594.
Lakshmi, B.V.S. and Divya, V. 2014. Antiurolithiatic and antioxidant activity of *Zingiber officinale* rhizomes on ethylene glycol-induced urolithiasis in rats. Int. J. Adv. Pharm. Med. Bioal. Sci. 2(3): 148–153.
Macneil, F. and Bariol, S. 2011. Urinary stone disease - assessment and management. Aust. Fam. Physician. 40(10): 772–5.
Makasana, A., Ranpariya, V., Desai, D., Mendpara, J. and Parekh, V. 2014. Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in rats. Toxicol. Rep. 1: 46–52.
Malini, M.M., Lenin, M. and Varalakshmi, P. 2000. Protective effect of triterpenes on CaOx crystal-induced peroxidative changes in experimental urolithiasis. Pharmacol. Res. 41: 413–418.
Marino, M. and Bersani, C. 1999. Antimicrobial activity of the essential oils of *Thymus vulgaris* L. measured using a bioimpedometric method. J. Food Prot. 62: 1017–1023.
Martin, N. and Nieto, V. 2011. Hypouricemia and tubular transport of uric acid. Nefrologia 31(1): 44–50.
Monika, J., Anil, B., Aakanksha, B. and Priyanka, P. 2012. Isolation, characterization and *in vitro* antiurolithiatic activity of cerpegin alkaloid from *Ceropegia bulbosa* Var. Lushii root. Int. J. Drug Dev. Res. 4(4): 154–160.
Mukerjee, P.K. 2002. Quality control of herbal drugs. 1st ed. New Delhi: Business Horizons Publication, pp. 2–24.
Munver, R. and Preminger, G. 2001. Urinary tract stones in comprehensive urology. St. Louis: Mosby.
Nickavar, B., Mojab, F. and Dolat-Abadi, R. 2005. Analysis of the essential oils of two thymus species from Iran. Food Chem. 90: 609–611.
Nolkemper, S., Reichling, J., Stintzing, F.C., Carle, R. and Schnitzler, P. 2006. Antiviral effect of aqueous extracts from species of the lamiaceae family against *Herpes simplex* virus type 1 and type 2 *In vitro*. Planta Med. 72: 378–1382.
Pareta, S.K. 2011. Establishing the principle of herbal therapy for antiurolithiatic activity: a review. J. Pharmacol. Toxicol. 6(3): 321–32.

Pareta, S.K., Patra, K.C., Mazumder, P.M. and Sasmal, D. 2010. *Boerhaavia diffusa* Linn. aqueous extract as curative agent in ethylene glycol induced urolithiasis. Pharmacol. Online. 3: 112–12.

Patel, R.R. and Mandal, S.D. 2014. Activity of *Withania somnifera* in ethylene glycol induced urolithiasis in rats. Int. J. Pharm. Arch. 3(3): 346–355.

Pathak, R.S. and Hendre, A.S. 2015. *In vitro* antiurolithiatic activity of *Kalanchoe pinnata* extract. Int. J. Pharmacog. Phytochem. Res. 7: 275–279.

Patil, A.S., Kale, A.S. and Paikrao, H.M. 2014. Screening the *in vitro* calcium oxalate crystal inhibition potential of *Abutilon indicum* L.: a common weed plant from the Indian medicine system. Austin. J. Biotech. Bioeng. 1(6): 4.

Patil, A.S., Paikrao, H.M., Kale, A.S. and Manik, S.R. 2015. Effect of aqueous extracts from therapeutic plants from indigenous ayurvedic system on *in vitro* calcium oxalate crystallization in human urine. Curr. Trad. Med. 1(3): 193–202.

Patil, A.S., Paikrao, H.M., Kale, A.S. and Manik, S.R. 2017. A TLC-Direct bioautography method for detection of antiurolithiatic metabolites. J. Chromatogr. Sci. 55(5): 578–585.

Pearle, M.S., Calhoun, E.A. and Curhan, G.C. 2005. Urologic diseases in America project: urolithiasis. J. Urology 173(3): 848–57.

Perez, C. and Anesini, C. 1994. Antibacterial activity of alimentary plants against *Staphylococcus aureus* growth. Am. J. Chin. Med. 22: 169–174.

Rajeshwari, P., Rajeswari, G., Jabbirulla, S.K. and Vishnu vardhan, I. 2013. Evaluation of *in vitro* antiurolithiasis activity of *Convolvulus arvensis*. Int. J. Pharm. Pharm. Sci. 5(3): 599–601.

Ramesh, M.S.K. and Shanmugam, R. 2014. Nephroprotective activity of Ethanolic Extract of *Orthosiphon stamineus* Leaves on Ethylene Glycol induced Urolithiasis in Albino Rats. Int. J. Pharm. Tech. Res. 6(1): 403–408.

Rathod, V.D., Fitwe, P., Sarnaik, D. and Kshirsagar, S.N. 2013. *In vitro* Anti-urolithiatic activity of corn silk of *Zea mays*. Int. J. Pharm. Sci. Rev. Res. 21(2): 16–19.

Reddy, N.M. 2013. *Lantana camara* Linn. Chemical constituents and medicinal properties: a review. Scholars Acad. J. Pharm. 2(6): 445–448.

Saranya, R. and Geetha, N. 2014. Inhibition of Calcium oxalate (CaOx) crystallization *in vitro* by the extract of Beet root (*Beta vulgais* L.) Int. J. Pharm. Pharm. Sci. 6(2): 361–365.

Sathyaa, M., Kokilavani, R., Ananta Teepa, K.S. and Balakrishnan, A. 2011. Supplementation of ethanolic extract of *Acalypha indica* Linn. prevents hyperoxaluria in experimental urolithic rats. J. Bloomers. Res. 4: 305–308.

Satish, H., Raman, D., Kshama, D., Shivananda, B.G. and Shridhar, K.A. 2009. Study the relative effect of spironolactone and different solvent extract of *Tribulus terrestris* on urolithiatic rats. Phcog. Mag. 5: 83–9.

Schneider, H.J., Bothor, W., Berg, W., Börner, R.H. and Jakob, M. 1983. A gel model for measuring crystallization inhibitor activities. Urol. Int. 38: 33–38.

Soundararajan, P., Mahesh, R., Ramesh, T. and Begum, V.H. 2006. Effect of *Aerva lanata* on Calcium Oxalate Urolithiasis in Rats. Indian J. Exp. Biol. 44(12): 981–986.

Spirnak, J.P. and Resnick, M.I. 1987. Retrograde percutaneous stone removal using modified Lawson technique. Urology 30(6): 551–3.

Stamatelou, K.K., Francis, M.E., Jones, C.A., Nyberg, L.M. and Curhan, G.C. 2003. Time trends in reported prevalence of kidney stones in the United States: 1976–1994. Kidney Int. 63: 1817–23.

Suzuki, K., Kawamura, K. and Tsugawa, R. 1999. Formation and growth inhibition of calcium oxalate crystals by Takusha (*Alismatis rhizoma*). Scan. Microscopy 13(2-3): 183–189.

Szczepanik, M., Zawitowska, B. and Szumny, A. 2012. Insecticidal activities of *Thymus vulgaris* essential oil and its components (Thymol and Carvacrol) against larvae of lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). Allelopath. J. 30: 129–142.

Thirumala, K., Janarthan, M. and Firasat, A.M. 2013. The aqueous extract of stem core of *Musa paradisiaca* against ethylene glycol and ammonium chloride induced urolithiasis. Int. J. Res. Pharm. Biotech. 1(6): 866–868.

Tina, C., Najumadeen, N.A.H., Kumar, S.V., Mainikandan, N., Tobin, G.C., Venkataraman, S. and Murugesh N. 2006. Antilithiatic effect of *Melia azedarach* on ethylene glycol induced nephrolithiasis in rats. Pharm. Bio. 44(6): 480–485.

Venilla, V. and Mariyal, A. 2015. *In vitro* analysis of phytochemical and antiurolithiatic activity of various extract of *Melia dubia* leaves. World J. Pharm. Pharm. Sci. 4: 1277–1289.

Venkatesh, G., Baburao, K., Rajesh, B.M., Dhanalakshmi, S. and Indira, P.D.G. 2010. The *Coleus aromaticus* activity. Int. J. Phytomed. 2: 284–291.

Vijaya, T., Rao, N.V.R., Narendra Babu, A., Kumar, S.M., Nirojini, P.S., Reddy, B.S. and Nadendla, R. 2013. Antiurolithiatic activity of methanolic extract of dried leaves of *Glochidionc velutinium* using ethylene glycol induced rats. Int. J. Biol. Pharm. Res. 4(12): 878–884.

Vyas, B., Vyas, R.B., Joshi, S. and Santani D. 2011. Antiurolithiatic Activity of Whole-Plant Hydroalcoholic Extract of *Pergularia daemia* in Rats. J. Young Pharm. 3(1): 36–40.

Wittstock, U. and Gershenzon, J. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. Curr. Opin. Plant. Biol. 5(4): 300–7.

Yamaguchi, S., Wiessner, J.H., Hasegawa, A.T., Hung, L.Y., Mandel, G.S. and Mandel, N.S. 2005. Study of a rat model for calcium oxalate crystal formation without severe renal damage in selected conditions. Int. J. Urol. 12: 290–298.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Section II

Ethnopharmacology

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

14

In Vitro Experimental Design and Data Analysis in Ethnopharmacology

Raúl Vinet,[1,2,*] *Marcela Knox,*[1] *Marcos Lorca-Morales,*[1] *Claudio Laurido*[3] and *José Luis Martínez*[4]

Introduction

Ethnopharmacology is an interdisciplinary field that seeks to understand the traditional therapeutic use of natural bioactive products. This area was first defined as "the interdisciplinary scientific exploration of active biological agents traditionally employed or observed by man" (Holmsted and Bruhn 1983). The term "ethnopharmacology" is an amalgam of the Greek word *ethno*s (culture), *pharmakos* (drug or medicine), and *logia* (the study of). On this basis, ethnopharmacology is considered as the study of drugs that have been traditionally used to treat ailments.

Ethnopharmacology pursues to understand the pharmacological basis of culturally significant plants, so that it may require preclinical and clinical pharmacological studies. It is common for a medicinal plant to have more than one use in a culture and even between cultures, a situation that makes its study even more challenging (Heinrich and Jäger 2015). Therefore, ethnopharmacology needs a multidisciplinary work approach that integrates scientific data offered by a variety of disciplines such as cultural anthropology, archeology, linguistics, history, botany, zoology, chemistry, pharmacology, toxicology, and medicine to take up the challenge (de Smet and Rivier 1989).

According to the World Health Organization (WHO) definition, there are three kinds of herbal medicines: raw plant material, processed plant material, and medicinal herbal products. Herbal drugs are defined as finished labeled medicinal products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations (Choudhary and Sekhon 2011).

Herbal and other traditional pharmacologic therapies are in widespread use throughout the world. A WHO estimation indicates that about 80% of the world population still uses herbs and other traditional medicine for their primary health care needs (Atmakuri and Dathi 2010). Such widespread use suggests but does not assure, that traditional medicines have a favorable risk-benefit ratio. The actual benefits and

[1] Laboratory of Pharmacology, Faculty of Pharmacy, Universidad de Valparaíso, Valparaíso 2360102, Chile.
[2] Regional Centre for the Study in Foods and Health (CREAS), Valparaíso 2362696, Chile.
[3] Faculty of Chemistry and Biology, Universidad de Santiago de Chile, Santiago 9170022, Chile.
[4] Vice Rectory of Research, Development and Innovation, Universidad de Santiago de Chile, Santiago 9170022, Chile.
* Corresponding author: raul.vinet@uv.cl

risks remain to be evaluated by clinical trials involving four sets of issues: chemical-manufacturing-control issues, non-clinical issues, clinical issues, and ethical issues. Two unique characteristics of herbal products are that they are multi-component mixtures and that substantial prior human use precedes their formal investigation (WHO 2005).

As a part of one of the fundamental disciplines of ethnopharmacology, this chapter focuses on pharmacology of medicinal plants. A distinctive feature of pharmacology is that the effect of a drug is often observed indirectly, which means that while a drug affects a biochemical process in a cell, the response is a large-scale change in the state of the whole body (Kenakin 2017). Accordingly, if the response is appropriately quantified it can be used to predict the effect of a drug at the pharmacological target in all systems including the therapeutic system.

Evaluation of bioactivities

Evaluation of bioactivities of natural products, including plant material, is not an easy task, especially if the research is in the field of ethnopharmacology. There are several mandatory steps to achieve consistent results. As shown in Fig. 14.1, the first step is to obtain reliable ethnopharmacological evidence indicating that a population use or has used a plant species for therapeutic purposes. Secondly, we must differentiate two parallel paths with their respective components and associated restrictions: Plant material and biological model.

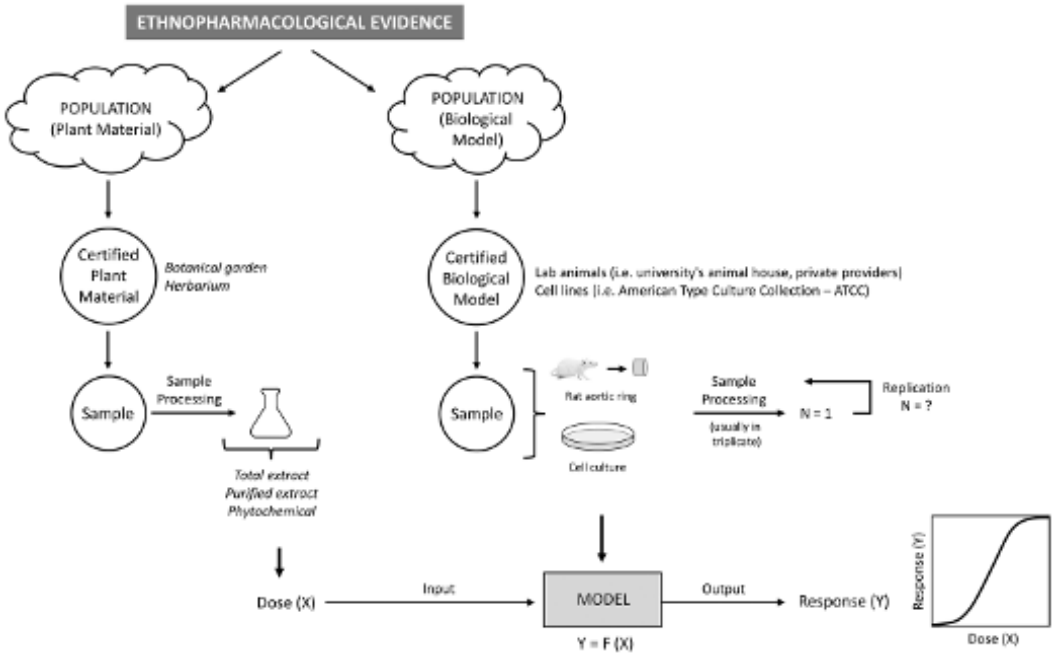


Fig. 14.1 A general approach to evaluate bioactivity of plant material (see the text for description).

Plant material

The "Plant Material" path considers the following components (C_1–C_4):

- **C_1.** Population (Plant Material). A theoretical population of plant species associated with the therapeutic effect.
- **C_2.** Certified Plant Material. A certified source of the "Population" (C_1), namely the means and sources of plant identification, the institutions holding the voucher specimens, and the experts that have helped to identify the sample (Weckerle et al. 2017).

- **C_3.** Sample. A sample of the "Certified Plant Material" (C_2), namely a representative sampling of the certified plan material (whole plant, roots, stem, leaf, flower, fruit, seed).
- **C_4.** Sample Processing. Typically, a dried and powdered plant material containing one or more plant parts. This material is usually macerated in water or ethanol-water at room temperature for some days. Then the material is filtered, evaporated *in vacuo* and lyophilized, obtaining the dry aqueous or hydroalcoholic extract. This product is the "total or whole extract" and the first source for bioactivity evaluation. Additional processes included fractionation and purification, finalizing with one or more isolated compounds. Additionally, these sources require different techniques to identify and quantify their components (Trease and Evans 1989, Lakshmana Prabu and Suriyaprakash 2012). In Fig. 14.1, these sources of bioactive material are represented by "Dose (X)".

Solution Preparation. A practical rule of thumb for preparing a drug stock solution is to weigh 10% of the drug's molecular weight and then dissolve it in 10 mL. For example, if a drug has a molecular weight of 200 Da, we can weigh 20 mg (or less) and then dissolve it in a total volume of 10 mL (or the equivalent volume depending on the mass weighed). Therefore, the final concentration will be 10^{-2} M (i.e., 10,000 μM).

In the following example, we will assume that we have a 10^{-2} M drug stock solution with the drug dissolved in pure ethanol. We also suppose that the final experimental concentration is defined as 100 μM (the highest concentration). The experimental chamber is a cell culture dish of 1 mL (i.e., 1,000 μL). Consequently, the experimenter adds 10 μL of stock solution into the dish and completes its volume to 1 mL with the experimental solution (e.g., saline solution or culture medium). Because of this procedure, the cell culture dish has reached 1% of ethanol.

If the biological model is highly sensitive to ethanol and 1% may be harmful, it is possible to prepare a more concentrated drug stock solution. This example is also valid for dimethylsulfoxide (DMSO), a solvent widely used in pharmacological tests for its ability to dissolve water-insoluble drugs.

Another important consideration relates to the stability of compounds derived from natural sources. Bioactive compounds present in plants are especially sensitive to temperature, pH, and light, so precautions must be taken to avoid degradation.

Biological model

The "Biological Model" involves the following components (C_5–C_8):

- **C_5.** Population (Biological Model). A theoretical population of the biological model suitable to evaluate the therapeutic effect associated with the plant species.
- **C_6.** Certified Biological Model. A certified source of the "Population" (C_5), specifically a biological model that have been proven to render the relevant pharmacological parameters that characterized a drug (Peng and Zhao 2009, Kenakin 2017). Usually, the certified biological model included laboratory animals and cell culture providers from universities and private organizations. At this point, we are going to focus on two biological models widely used *in vitro* assays: rat aortic rings and Human Umbilical Vein Endothelial Cells (HUVEC).
- **C_7.** Sample. A sample of "Certified Biological Model" (C_2), namely a representative sample of the certified biological model (laboratory animals, cell cultures).
- **C_8.** Sample Processing. *In vitro* (e.g., primary cultures, cell lines, isolated organs) and *in vivo* (laboratory animals like mouse and rat) models play a significant role in preclinical pharmacological assays (Peng and Zhao 2009). In this work, we described two *in vitro* models, rat aortic rings, and HUVEC:

Rat Aortic Ring Preparation. We will describe the modified methods for measuring rat aorta contractility (Vinet et al. 1991, Illanes et al. 1993, Vinet et al. 2012). Rats weighing 250–290 g are sacrificed by an overdose of CO_2 inhalation. The thoracic aorta is carefully excised and placed in a Petri dish containing modified Krebs-Henseleit buffer (KHB) (in mM: NaCl 122; KCl 4.7; $NaHCO_3$ 15.5; KH_2PO_4 1.2; $MgCl_2$ 1.2; $CaCl_2$ 2.0; D-glucose 11.5; EDTA 0.026; pH 7.4) at 37°C and oxygenated continuously with a 95% O_2 – 5% CO_2 gas mixture. Aorta is dissected, cleaned of connective tissue and divided into 5 mm rings segments. Rings are suspended between two L-shaped stainless-steel hooks and placed into a 20–30 mL organ chambers containing modified KHB at 37°C and oxygenated continuously with a 95% O_2 – 5%

CO_2 gas mixture. Isometric tensions are measured using a force displacement transducer connected to a polygraph. Rings are exposed to a basal tension of 1.5 g for 60 minutes. Then, rings are progressively stretched at least three times with a depolarizing 70 mM KCl solution (in mM: NaCl 52; KCl 70.0; $NaHCO_3$ 15.5; KH_2PO_4 1.2; $MgCl_2$ 1.2; $CaCl_2$ 2.0; D-Glucose 11.5; EDTA 0.026; pH 7.4) until reaching a maximum stable contraction (reference tension). Rings are repeatedly washed and equilibrated for 30 minutes. The preparation is now ready to evaluate aorta contractility, i.e., relaxation or contraction activities. The analysis of the effect of a drug on aortic reactivity included the maximal response and the concentration causing 50% of the maximal response (EC_{50}). The integrity of endothelium is assessed by testing the relaxation produced by the addition of 1 μM acetylcholine in 0.1 μM phenylephrine-precontracted rings.

To evaluate a drug with a vasoconstriction potential, the drug is added directly to the organ chamber containing the aorta under a basal tension. On the other hand, if a drug is suspected to have a vasodilatory potential, the vessel is previously contracted with phenylephrine, and when a stable contraction is reached, the drug is added. In both cases, the drug is applied in increasing doses, usually between concentrations from 10^{-9} to 10^{-4} M.

HUVEC Preparation. Primary culture of HUVEC are isolated and pooled from umbilical cords obtained from normal vaginal deliveries according to Jaffe et al. (1973) and modified by Cortés et al. (2013). Cells are cultured in gelatin-coated Petri dishes and grown in medium 199 supplemented with 2 mM glutamine, 20% heat-inactivated fetal bovine serum, and 25 mg/mL endothelial cell growth supplement. HUVEC are incubated in 5% CO_2–95% air-gas mixture. The medium is changed 24 hours after seeding and cells subcultured on reaching confluence using 0.01% trypsin-EDTA. Usually, HUVEC of the first and second passage is used for experiments. Cells are seeded at a density of (1.3×10^5 cell/mL) in gelatin-coated Petri dishes and allowed to attach overnight. HUVEC are ready to be submitted to the experimental protocol.

Experimental design and data analysis

Statistical Analysis. The randomized and blinded controlled experiment was first established in the early 1900s by Fisher in agricultural research (Festing and Nevalainen 2014). The rationale is clear: if the test compares an intervention in two or more groups, necessarily need to be contrasted with a non-intervention group, i.e., the control group. If the researchers know the treatments, they can bias the results favoring one group. In blinded, controlled experiment it is the statistical analysis that determines the probability that differences among groups can be attributed to the effect of the intervention rather than chance.

Replicates are intended to evaluate and isolate sources of variation in measurements and to limit the effect of false variation on parameter estimation. Distinguishing between biological and technical variation is important. Biological variation can be detected by measurements of distinct samples while technical variation can be detected by repeated measurements of the same sample. Taking additional biological replicates is the best option for improving the efficiency of statistical testing (Blainey et al. 2014).

Our experience shows that by working with calibrated instruments, with triplicate samples, and with six independent experiments (N = 6), it is possible to obtain consistent results allowing us to discriminate small effects attributable to the intervention being evaluated.

Mathematical Models. The purpose of this section is to find a simple model to adjust the experimental data and to obtain the parameters that characterized the system. A system is a pool of objects and processes that interact to create a unified whole, such as a cell culture system, a rat, or a human (Bonate 2011). A mathematical model is a caricature, a deliberate oversimplification of reality (Hannon and Ruth 2014). Therefore, a mathematical model represents the system of interest and can be used to explore its structure and behavior (Wastney et al. 1997).

The regression model is an equation that defines the Output, or dependent variable Y, as a function of the Input, or independent variable X, and one or more model parameters (Motulsky and Christopoulos 2003). There are two types of regression models, empirical and mechanistic models. Empirical models just describe the general form of data that is tried to fit. The parameters of the model do not necessarily correspond to a natural process.

On the other hand, mechanistic models are explicitly formulated to represent a specific natural process that is supposed to control the phenomenon under study. The parameters resulting from mechanical models

are quantitative estimates of real system properties. In general, the mechanistic models are more useful because they are a quantitative representation of a hypothesis. However, if the wrong mechanistic model is chosen to fit the data, the consequences are more negative than for the empirical models, since it is very likely that erroneous conclusions are reached regarding the mechanism under study (Motulsky and Christopoulos 2003).

Dose-Response Curves. Dose-response curves are used to plot the results of many types of biological experiments mainly pharmacological. The X-axis represents the concentration of a bioactive compound (e.g., drug, hormone). The Y axis represents the biological response (e.g., enzymatic activity, intracellular messenger level, contraction, etc.). Figure 14.2 shows a representation of a dose-response curve as a cell process.

The term "dose" is frequently used to refer to both *in vivo* and *in vitro* assays (dose-response curve). However, to be fair, it should be clarified that the term dose is suitable for *in vivo* experiments (i.e., dose-response curve), whereas for *in vitro* experiments it is more correct to use concentration (i.e., concentration-response curve). It is important to consider that in *in vivo* experiments the concentration of drug at the site of action is unknown so that only dose control is available. In contrast, *in vitro* experiments, it is generally assumed that the concentration of drug at the site of action is equal to the concentration of drug reaching the system and therefore is controlled by the investigator.

An agonist is a drug that binds to a receptor and alters the receptor state triggering a biological response. If several concentrations of an agonist induce a sharp response, the dose-response curve will go uphill as drug concentration increases. On the other hand, if the agonist produces an inhibitory response, the curve will go downhill as drug concentration increases. A full agonist is a drug producing the maximum cellular or tissue response. A partial agonist is a drug that provokes a response, but the maximum response is less than the maximum response compared to a full agonist in the same cell or tissue. An inverse agonist is a drug that decreases an established basal response.

An antagonist is a drug that decreases the action of another drug, generally an agonist. It is important to note that the classification of drugs as full agonists, partial agonists, inverse agonists, and antagonists is highly dependent on the biological system in which they are tested.

Choosing the Mathematical Model: To fit data obtained from pharmacological experiments we suggest using the standard model known as the "Hill equation", the "four-parameter logistic equation", the "variable slope sigmoid equation" or simply "4PL" (Motulsky and Christopoulos 2003). This model can be written

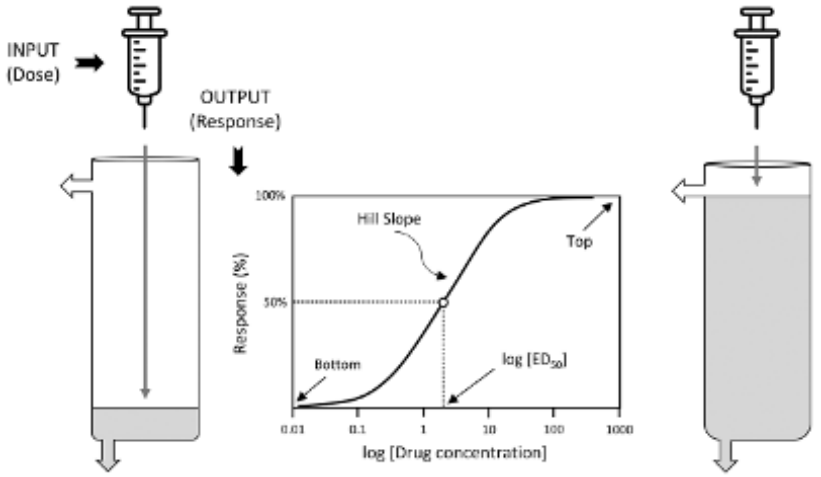


Fig. 14.2 Representation of a dose-response curve as a cell process. The figure shows a system symbolized by the sequence INPUT-PROCESS-OUTPUT. The amount of liquid entry into the container (e.g., fuel) represents the INPUT (Dose), and the level of liquid in the container (e.g., energy) represents the OUTPUT (Response). The Bottom level corresponds to the basal level and the Top level the maximal effect. Note that the INPUT (Dose) is transformed into OUTPUT (Response) by a PROCESS that can be represented by a mathematical model. A key task of pharmacology is to find the model and associated parameters that allow characterizing a drug and inferring its action in other biological systems, particularly in human beings.

as an equation that defines the response (the dependent variable Y) as a function of dose (independent variable, X) and the four parameters as shown in Fig. 14.2:

$$Y = \text{Bottom} + \frac{\text{Top - Bottom}}{1 + \left(\frac{10^{\text{LogEC}_{50}}}{10^{X}}\right)^{\text{Hill Slope}}}$$

where,

Bottom = Y value for the minimal curve asymptote (theoretically, the response in the absence of drug)

Top = Y value maximal curve asymptote (theoretically, the response produced by an infinitely high concentration of drug).

$LogEC_{50}$ = the logarithm of drug dose (or concentration) that produces the response halfway between the Bottom and Top (commonly used as a measure of a drug's potency).

Hill Slope = indicates the steepness of the dose-response curve and usually associated with the system's sensitivity to the drug.

The next step is defining which of the parameters, if any, should be fixed to constant values. This decision should be based on experimental controls, or on theoretical aspects. When the basal response of the system is assumed to be zero, it is often to consider the Bottom value also as zero.

Nonlinear regression can be performed using GraphPad Prism (GraphPad, San Diego, CA, USA) and SigmaPlot (Systat Software, Inc., San Jose, CA, USA). Both programs have a trial version.

Conclusion

Ethnopharmacology pursues to understand the traditional therapeutic use of natural bioactive products. To reach this purpose ethnopharmacology needs a multidisciplinary work approach that integrates scientific data offered by a variety of disciplines including pharmacology. In this work, we conclude that to achieve reliable data that understand and validate the traditional use of bioactive compounds, a strict experimental protocol aligned with the scientific method is necessary. To contribute to experimental research in ethnopharmacology, in this chapter, we have proposed a general pharmacological approach to obtain an acute estimation of plant material bioactivity using two *in vitro* models: isolated rat aorta and endothelial cell cultures.

References

Atmakuri, L.R. and Dathi, S. 2010. Current trends in herbal medicines. J. Pharm. Res. 3: 109–13.
Blainey, P., Krzywinski, M. and Altman, N. 2014. Points of Significance: Replication. Nat. Methods 11: 879–s880.
Bonate, P.L. 2011. Pharmacokinetic-Pharmacodynamic Modeling and Simulation. 2nd ed. Springer, USA.
Choudhary, N. and Sekhon, B.S. 2011. An overview of advances in the standardization of herbal drugs. J. Pharm. Educ. Res. 2: 55–70.
Cortés, M.P., Becerra, J.P., Vinet, R., Alvarez, R. and Quintana, I. 2013. The inhibition of ATP-induced calcium influx by homocysteine in human umbilical vein endothelial cells. Cell Biol. Int. 37: 600–607.
De Smet, P.A. and Rivier, L. 1989. A general outlook on ethnopharmacology. J. Ethnopharmacol. 25: 127–138.
Festing, M.F. and Nevalainen, T. 2014. The design and statistical analysis of animal experiments: introduction to this issue. ILAR J. 55: 379–382.
Hannon, B. and Ruth, M. 2014. Modeling Dynamic Biological Systems. 2nd. ed. Springer, USA, pp. 3–10.
Heinrich, M. and Jäger, A.K. 2015. Ethnopharmacology. 1st ed. Wiley Blackwell, Oxford, UK, pp. 3–5.
Holmstedt, B. and Bruhn, J. 1983. Ethnopharmacology—a challenge. J. Ethnopharmacol. 8: 251–256.
Illanes, A.G., Penna, M. and Martinez, J.L. 1993. The vascular relaxing effect of a factor released by rat atria distension. Gen. Pharmacol. 24: 315–319.
Jaffe, E.A., Nachman, R.L., Becker, C.G. and Minick, C.R. 1973. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J. Clin. Invest. 52: 2745–2756.
Kenakin, T.P. 2017. Pharmacology in drug discovery and development: understanding drug response. 2nd. ed. Amsterdam: Elsevier/Academic Press, pp. 7–40.
Lakshmana Prabu, S. and Suriyaprakash, T.N.K. 2012. Extraction of drug from the biological matrix: a review. *In*: Ganesh R. Naik (ed.). Applied Biological Engineering - Principles and Practice. ISBN: 978-953-51-0412-4, InTech.

Motulsky, H.J. and Christopoulos, A. 2003. Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. GraphPad Software Inc., San Diego CA, USA, pp. 256–265.

Peng, S. and Zhao, M. 2009. Pharmaceutical Bioassays: Methods and Applications. John Wiley & Sons, Inc., Hoboken, New Jersey, USA, pp. 1–443.

Trease, G.E. and Evans, W.C. 1989. Pharmacognosy. 13th ed. Bailliere Tindall, London, UK.

Vinet, R., Brieva, C., Pinardi, G. and Penna, M. 1991. Modulation of alpha-adrenergic-induced contractions by endothelium-derived relaxing factor in rat aorta. Gen. Pharmacol. 22: 137–142.

Vinet, R., Knox, M., Mascher, D., Paredes-Carbajal, C. and Martinez, J.L. 2012. Isolated aorta model and its contribution to phytopharmacology. Bol. Latinoam. Caribe Plant. Med. Aromat. 11: 35–45.

Wastney, M.E., Siva Subramanian, K.N., Broering, N. and Boston, R. 1997. Using models to explore whole-body metabolism and accessing models through a model library. Metabolism 46: 330–332.

Weckerle, C.S., de Boer, H.J., Puri, R.K., van Andel, T., Bussmann, R.W. and Leonti, M. 2017. Recommended standards for conducting and reporting ethnopharmacological field studies. J. Ethnopharmacol. 210: 125–132.

World Health Organization. 2005. Operational Guidance: Information Needed to Support Clinical Trials of Herbal Products. (Document reference who/TDR/GEN/Guidance/051). http://apps.who.int/iris/bitstream/10665/69174/1/TDR_GEN_Guidance_05.1_eng.pdf.

15

Insights into the Ethnopharmacological Uses of Rutaceae

A Golden Source for Many Pharmaceutical Preparations

Dalia I. Hamdan,[1] *Fadia S. Youssef,*[2] *Mohamed L. Ashour*[2,*] and *Assem M. El-Shazly*[3]

Introduction

The family Rutaceae that is also named the Rue or *Citrus* family belongs to the order Sapindales. Most species of the family Rutaceae are mainly herbs, trees as well as shrubs, having a particular glandular punctuate in addition to possessing a strong scent. It includes almost 150 genera with 1,500 species that are greatly spread worldwide with special occurrence in tropical as well as subtropical regions particularly in southern Africa and Australia (Hume 1957, Reuther et al. 1967, Mabberley 2008).

In spite of the existence of numerous subfamilies within the family Rutaceae, all the members of genus *Citrus* belong to the subfamily Aurantioideae, that comprises of two tribes namely, the Clauseneae and Citreae, the former includes the very remote citroid fruit trees with five genera. While the latter comprises of citroid fruit trees with three subtribes: the first is the Triphasiinae that is named by minor citroid fruit trees; the second is the Balsamocitrinae that is also known by hard–shelled citroid fruit trees whereas the third is Citrinae (*Citrus* fruit trees) (Ortiz 2002, Mabberley 2008). The above taxonomy is illustrated in Fig. 15.1.

The *Citrus* family is very popular by the presence of spines in addition to winged petioles. Additionally, the leaves are characterized by being exstipulate, opposite or alternate in its phyllotaxis that are simple or pinnately or palmately or compound. However in some cases it is characterized by being in the form of sheath like or diminished to spines. Meanwhile, most flowers of the Rutaceae members possess a characteristic sweet odor and are mostly bisexual, and actinomorphic and occasionally zygomorphic. Basically, its calyx contains 3–5 sepals that are either discriminate or basally connate meanwhile its corolla comprises of 3–5 petals that are mostly distinct and sometimes connate and scarcely the petals are

[1] Department of Pharmacognosy, Faculty of Pharmacy, Menoufia University, 32511 Shebin El Koum, Egypt.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, 11566, Cairo, Egypt.
[3] Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519 Zagazig, Egypt.
* Corresponding author: mohamed_ashour@pharm.asu.edu.eg; ashour@pharma.asu.edu.eg

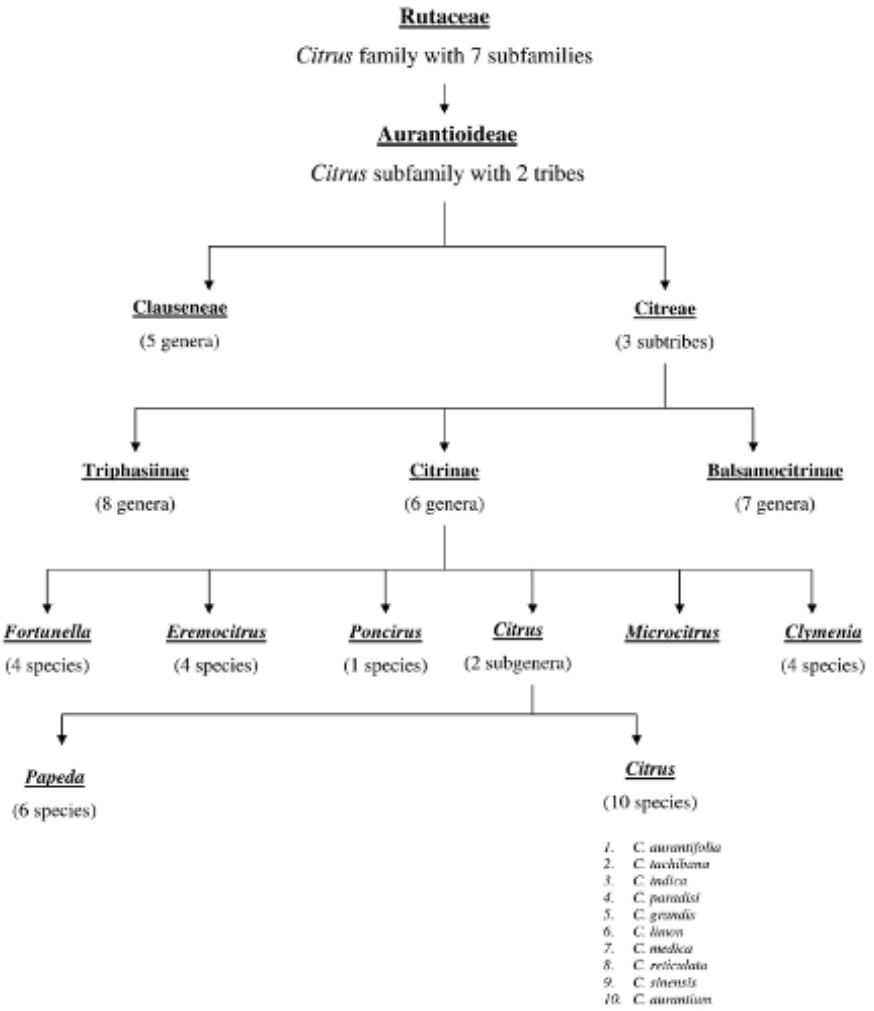


Fig. 15.1 Phylogenetic tree of Rutaceae family.

absent. Besides, the fruit is extremely variable in its appearance as well as in its size. The former ranges from round to sub-globose as well as oblate to obovoid and from broadly pyriform to cylindrical, ovoid, elliptical and oblong. Furthermore, its rind is familiar by being very variable, rough, ribbed, wrinkled or bumpy. The variation in the internal characters between the fruits of *Citrus* members mainly relied upon the discriminate structure of hesperidium in addition to the fruit segments and inclusions, the rind, the central axis or medulla, and the seeds (Ortiz 2002, Mabberley 2008). In the foregoing chapter we aim to shed light on the traditional uses, phytochemistry and its correlation with the biological activity of some important species of one of the popular genera in the Rutaceae that is the genus *Citrus*.

Genus *Citrus*

Citrus is among the most important genera of flowering plants within the Rutaceae, that is native to tropical, semitropical as well as subtropical Southeast Asia. The term *Citrus* originates from the Ancient Greek word that is kedros or from its Latin derivate which is called cedrus. It is noteworthy to mention, that the names were applied by both the ancient Greeks as well as by the Romans describing various trees that possess fragrant foliage or wood (Andrews 1961, Ortiz 2002).

The genus *Citrus* is characterized by a very complex taxonomy and the actual figure of the naturally occurring species is still unclear in addition to the occurrence of many clonally-propagated hybrids with genetic evidence that declares that even the wild, true–breeding species are of hybrid origin. True fruits of *Citrus* comprise five genera and 29 species that are as follow: *Fortunella* that includes four species, *Eremocitrus* with four species also, in addition to *Poncirus* that is of only one species whereas, *Citrus* includes two subgenera and 16 species, *Microcitrus*, and *Clymenia* that possess four species.

Moreover, the two discriminate subgenera of the genus *Citrus*, *Citrus* and *Papeda*, are easily differentiated by the characters of their leaf, flower, as well as the fruit. Most of the popular cultivated members of *Citrus* exemplified by mandarin, sweet orange, lemon, lime and grapefruit are contained within the subgenus *Citrus* (Fig. 15.2). These species are characteristic by the presence of pulp vesicles which are filled with a pleasant acidic or sweet juice with the presence of an everlasting unifoliate or simple leaves (Ortiz 2002, Mabberley 2008).

Citrus fruits are considered not only as a favorite food but also a medicinally active agent possessing multiple phytoconstituents that are beneficial to human health. These constituents are mainly limonoids, flavonoids, coumarins and acridone alkaloids in addition to the presence of a high level of vitamin C, vitamin–B complex as folic acid, biotin, pantothenic acid, pyridoxine and β–carotene and lycopene type carotenoids (Berhow et al. 2000, Ladaniya 2008). Notable improvement in blood circulation, anti-allergic, antiviral and anti-carcinogenic activities has been attributed to *Citrus* juices in particular to that of grapefruit and oranges owing to their richness by flavonoids. Furthermore, fresh *Citrus* fruits are also popular by reducing the risk of heart attacks if taken in the diet on a daily basis owing to their high content of fiber and pectin. The fragrance obtained from the flowers of grapefruits is effective in relieving insomnia and as a stomachic and cardiac tonic. The pulp provides an effective therapy in the alleviation of urinary disorders in addition to the antibiotic activity exhibited by leaf extracts (Filatova 1999).

Nowadays, *Citrus* is considered as the most widely spread and produced fruits in the form of as a group of numerous species that is grown in more than 80 countries (Chang 1992, Berhow et al. 2000). The production of *Citrus* in the globe elevated at the rate of 4.5% annually during the 1990s, that resulted in the production of 98.35 million tons during the period between 2001 and 2002, and this number exceeded the 100 million tons mark during 2003–2004 (FAO 2006).

Owing to the tremendous economic developments and rapid alteration in the lifestyle of people with different cultures, fresh fruit utilization is highly elevating in the category of easy–peelers, such as tangerines/mandarins. Being seedless and small, these fruits are more preferable and convenient in eating.

Fig. 15.2 Different fruits belonging to Citrus subgenus.

Moreover, consumption of fresh oranges rapidly increased by a rate of 2.9% from 1986–88 to 1996–98. In regions that are characterized by their high population such as African regions and many Asian countries as China and India, *Citrus* fruits are used in a fresh form and are supplied by local *Citrus* industries. In Central and Latin America, a large category of the population still prefers and can withstand the consumption of only fresh *Citrus*. The majority of the rise in the consumption of domestic production is mainly in China, India, Mexico, Pakistan, and Brazil. In spite of the increase in the production of *Citrus* fruits with a rate of 2–5% each year, the per capita annual consumption differs from 40 kg in European countries to 4 kg in Asia and Africa countries (Ladaniya 2008).

Traditional uses of family Rutaceae and genus *Citrus*

In the folk medicine *Citrus* plants were believed to be an effective method to combat moths in clothes as well as a mouthwash as written by Theophrastus owing to its insecticidal limonoids that were recently discovered. Moreover, it was popular as an efficacious poison antidote and as a tonic keeping the vitality of skin as mentioned by Abu Marwan in his 'Treatise of foodstuff'. Regarding the pharmaceutical importance of the *Citrus* plants, it was early recognized by Linnaeus. Additionally, sour orange was greatly adopted in the Middle Ages in Europe both as a flavoring agent and to prevent worms. Besides, it gained a great reputation in China to alleviate a wide array of disorders starting from mild skin ailments to influenza, gout, cough, sore throat, liver and gall bladder problems, rheumatism and to reduce blood pressure (Arias and Ramón-Laca 2005).

Additionally, in western medicine, the essential oil of *Citrus* plants were widely consumed as an antiseptic against bacteria, fungi, protozoa and insects attributing to their high content of phenol. *Citrus hystrix* that is familiar by the richness of its leaves with citronellal is recognized to be essential in cooking, meanwhile the hesperidium is still consumed to wash hair and to propel leeches. *Citrus* plants were widely utilized in the west to alleviate scurvy that is caused by significant reduction in vitamin C in addition to common cold (Arias and Ramón-Laca 2005).

Moreover, some clinicians in the early last century postulated that lemon juice countered obesity *via* the interaction that takes place between a compound existing in the fruit's 'membrane' and the liver to diminish cholesterol content and to control insulin. Additionally, it was reported that black women in tropical America utilize a douche comprising of lemon juice as a mean of contraception through its action as a potent spermicide in which the citric acid causes destruction of the proteins of the mitochondria resulting in immobility. Also, the pips were used during pregnancy to combat nausea and stomach weakness whereas lime pickle enhances the appetite. It is worth mentioning that control of HIV spread among the population was believed to be achieved via the consumption of lemon juice (Mabberley 2004).

Biological activity of genus *Citrus* and its correlation with its predominant classes of secondary metabolites

Biological activity of limonoids

Limonoids represent one of the major classes of active constituents in the *Citrus*, which is characterized by the attachment of a furan ring to C_{17} position that effectively induced glutathione S-transferase in both mice and rats. The effectiveness of a certain drug to enhance the activity of glutathione S-transferase has been notably correlated with its efficacy to inhibit carcinogenesis. The limonoids existing in the *Citrus* have been proved to inhibit the occurrence of the neoplasia that is induced by chemicals in the skin, fore-stomach, small intestine, colon and the lungs of experimental, laboratory animals. Also, they prohibited the proliferative spread of human breast cancer cell (Berhow et al. 2000). Besides, they enhance human health acting as cholesterol–lowering as well as antiviral agents (Manners 2007).

Furthermore, limonoids exhibited a marked inhibition concerning the growth of estrogen receptor-negative and positive human breast cancer cells culture. A mixture of limonoids consisting of limonin, nomilinic acid 17-β-glucoside, nomilin, and obacunone 17β-glucoside has shown an obvious inhibition in human breast cancer cell line (MCF-7). Additionally, a high concentration of 100 μg/ mL from the previously mentioned mixture could effectively induce apoptosis in MCF-7 (Tian et al. 2001).

The effect of *Citrus* limonoids as an antifeedant against insects was first discussed in 1982. It has been largely confirmed that limonoid aglycones showed an antifeedant activity towards a large number of insects comprising corn earworm, *Colorado potato* beetle, spruce budworm, fall armyworm and tobacco budworm. In addition, it was reported that previously mentioned limoniods showed insecticidal activity against *Culex quinquejasciatus* larvae (Jayaprakasha et al. 1997). The efficacy of limonin as antifeedant was found to be one tenth comparable to azadirachtin. Limonin as well as ichangensin exhibited significant antifeedant against *Colorado potato* beetle. On the contrary, limonoid glucosides did not show any antifeedant property against insects (Berhow et al. 2000, Ruberto et al. 2002).

Furthermore, it was proved that limonin showed hypoglycemic activity (Hasegawa et al. 1986) in addition to antitumor activity towards benzo[α] pyrene and azoxymethane induced neoplasia in the fore –stomach of mice and colon of rats, respectively (Lam and Hasegawa 1989, Tanaka et al. 2000, Tanaka et al. 2001). Additionally, limonin exhibited anti-inflammatory property towards paw edema induced by bradykinin and ear swelling produced by arachidonic acid (Matsuda et al. 1998).

Furthermore, a notable antifungal property was exhibited by limonin, nomilinic acid and limonol against *Puccinia arachidis*. The activity of limonoids is mainly due to the presence of a highly reactive epoxide group that is composed of three ring structures in addition to anoxirane ring that could effectively bind to proteins via covalent bonds to the sulfhydryl (SH), NH groups and the amino group (NH_2) of the amino acid residue. Consequently, this will probably lead to a significant alteration in both the conformation as well as the recognition processes of protein. Epoxides have long been known as powerful alkylating agents that can alkylate DNA resulting in mutations and malformations that consequently lead to cancer if not repaired by the naturally occurring repair enzymes (Govindachari et al. 2000, Wink 2008). Examples of some limonoids isolated from some members of genus *Citrus* is represented in Table 15.1.

Biological activity of flavonoids

Flavonoids also constitute one of the major classes of secondary metabolites predominating in *Citrus* that eventually showed a marked role in both human diet and health. In addition, flavonoids exhibited a great diversity in functions and actually attracted many researchers in attributing many reasons including ecological, biological and chemotaxonomic, functions and acting as discriminant markers assessing food quality as well as potential industrial investments (Tusa et al. 2007). Flavonoids prevailing in this genus are highly popular as anti-allergic, anticarcinogenic, antioxidant, antiviral and as a cardioprotective agent (Cook and Samman 1996, Del Caro et al. 2004, Lee et al. 2004, Yu et al. 2005). Besides, they showed a drastic effect in improving capillary fragility and inhibiting the aggregation of human platelet (Bocco et al. 1998).

In spite of the comprehensive studying of the biological activity of a large number of popular *Citrus* flavonoids (e.g., tangeretin, hesperidin, naringin and diosmin, and) both *in vivo* and *in vitro*, the potential biological activities of others as the hydroxycinnamates and several minor flavonoids have not been fully elucidated in *Citrus*. Meanwhile, hydroxycinnamates isolated from other food crops have been widely investigated and was proved to exhibit marked beneficial roles, comprising their actions as antioxidants, antimicrobial and anticancer agents (Manthey and Grohmann 2001).

In an attempt to assess the biological activities of *Citrus* flavonoids, naringin has showed a potential antihypercholesterolemic activity, it reduced the total cholesterol and low-density lipoprotein cholesterol levels in plasma (Jung et al. 2003), moreover, hesperetin and its related derivatives significantly lowered the total cholesterol and triglyceride concentration in plasma (Kim et al. 2003).

On the other hand, many other flavonoids including hesperidin, diosmin, quercetin and rutin act as a chemopreventive agent against colorectal carcinogenesis and neoplasia induced by azoxymethane (Tanaka et al. 2000, Kawaii et al. 1999). The polymethoxylated flavones in *Citrus* have also been shown to exhibit antiproliferative effects against many cell lines as well as anti-inflammatory actions (Manthey and Grohmann 2001). For instance, nobiletin down-regulated the production of pro-matrix metalloproteinase 3, 9 and to interfere with the proliferation of synovial fibroblasts (Ishiwa et al. 2000). Also, it showed amelioration in the hepatic parameters (lipid peroxides, reduced glutathione, and catalase enzyme) in mice (Wink 1999). Tangeretin showed a relevant suppressive effect on malignant tumor invasion and metastasis. Besides, treatment of ARPE-19 human retinal pigment epithelial cells with diosmin after being exposed to a high

Table 15.1 Examples of some limonoids isolated from some members of genus *Citrus*.

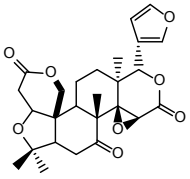
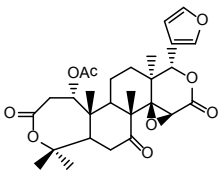
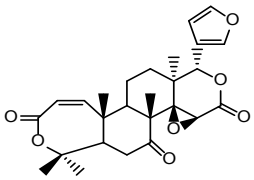
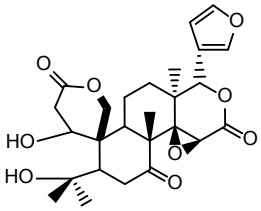
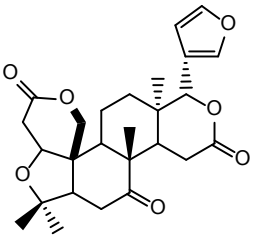
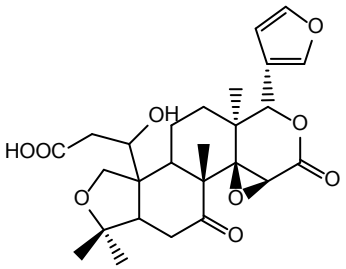
Compound	Structure	Formula, molecular weight	Source	Reference
Limonin		$C_{26}H_{30}O_8$ M^+ 470.3	*C. medica* *C. reticulata* *Calamondin** (*C. reticulata*+ *Fortunella* sp.) *C. limon*	(Cai et al. 1993) (Breksa and Ibarra 2007) (Manners and Breksa 2004) (Vikram et al. 2007)
Nomilin		$C_{28}H_{34}O_9$ M^+ 515.3	*C. medica* *C. reticulata* *Calamondin** *C. limon*	(Cai et al. 1993) (Vikram et al. 2007) (Manners and Breksa 2004) (Vikram et al. 2007)
Obacunone		$C_{26}H_{30}O_7$ M^+ 454.4	*Calamondin** *C. reticulata* *C. limon*	(Manners and Breksa 2004) (Khalil et al. 2003) (Moodley et al. 1995)
Ichangin		$C_{26}H_{32}O_9$ M^+ 488.53	*C. reticulata* *Calamondin** *C. limon*	(Khalil et al. 2003) (Manners and Breksa 2004) (Vikram et al. 2007)
Deoxylimonin		$C_{26}H_{30}O_7$ M^+ 454.51	*Calamondin** *C. limon*	(Manners and Breksa 2004) (Miller et al. 2004)
Isolimonic acid		$C_{26}H_{32}O_9$ M^+ 488.53	*C. reticulata* *C. limon*	(Vikram et al. 2007)

Table 15.1 contd....

...Table 15.1 contd.

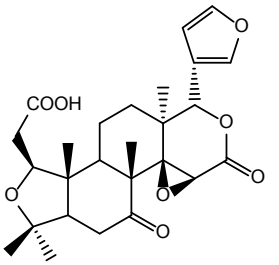
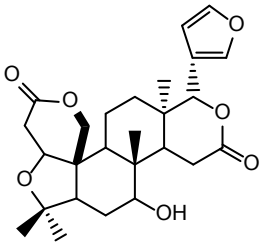
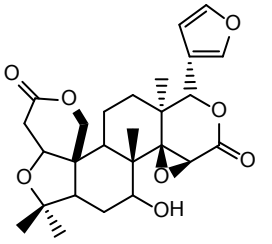
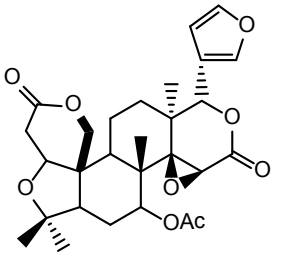
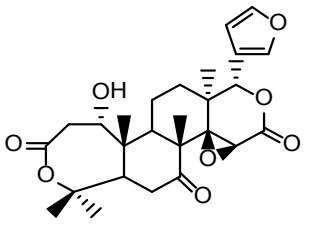
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Isoobacunoic acid		$C_{26}H_{32}O_8$ M^+ 472.53	*C. reticulata* *C. limon*	(Vikram et al. 2007)
Deoxylimonol		$C_{26}H_{32}O_7$ M^+ 456.53	*Calamondin**	(Manners and Breksa 2004)
7 α-Limonol		$C_{26}H_{32}O_8$ M^+ 472.53	*Calamondin** *C. limon*	(Manners and Breksa 2004) (Manners and Hasegawa 1999)
7α-Limonol acetate		$C_{28}H_{34}O_9$ M^+ 514.56	*Calamondin**	(Manners and Breksa 2004)
Deacetylnomilin		$C_{26}H_{32}O_8$ M^+ 472.4	*Calamondin** *C. reticulata* *C. limon*	(Manners and Breksa 2004) (Bennett and Hasegawa 1981) (Tian et al. 2003)

Table 15.1 contd....

...Table 15.1 contd.

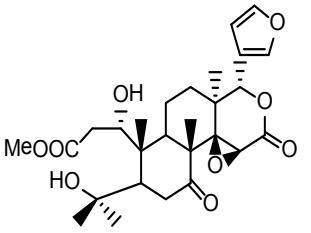
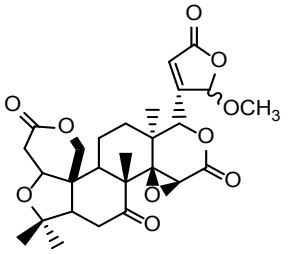
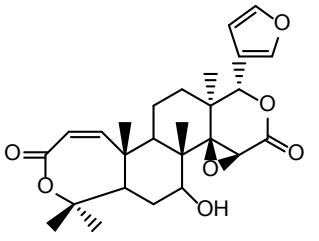
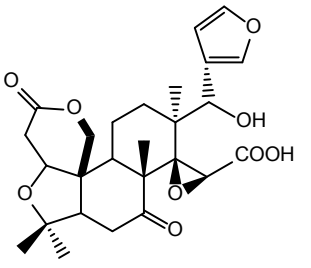
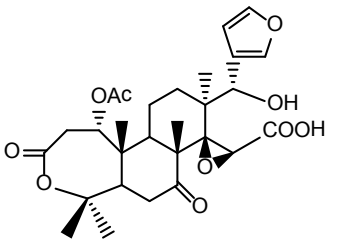
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Methyl deacetylnomilinate		$C_{27}H_{36}O_9$ M^+ 504.3	*Calamondin** *C. reticulata*	(Manners and Breksa 2004) (Bennett and Hasegawa 1981)
Isolimonexic acid methyl ether		$C_{27}H_{32}O_{10}$ M^+ 516.21	*C. reticulata*	(Khalil et al. 2003)
7 α-Obacunol		$C_{26}H_{32}O_7$ M^+ 456.3	*Calamondin**	(Manners and Breksa 2004)
Limonoic acid A-ring lactone		$C_{26}H_{32}O_9$ M^+ 488.53	*C. reticulata* *C. limon*	(Breksa et al. 2005) (Raman et al. 2005)
Nomilinoate A-ring lactone		$C_{28}H_{36}O_{10}$ M^+ 532.58	*C. reticulata* *C. limon*	(Breksa and Ibarra 2007) (Raman et al. 2005)

Table 15.1 contd....

...Table 15.1 contd.

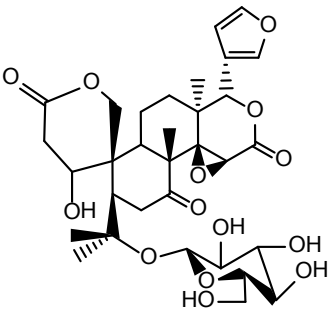
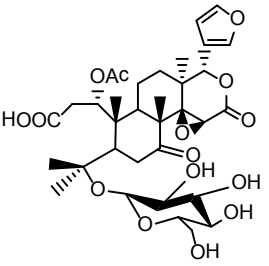
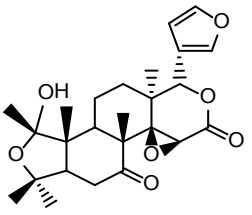
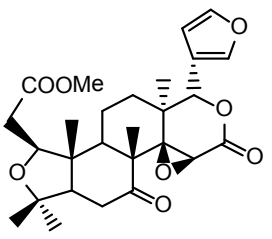
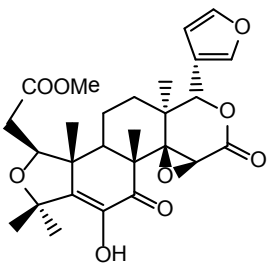
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Ichangin 4-β-glucoside		$C_{32}H_{42}O_{14}$ M^+ 650.1	*C. limon*	(Matsubara et al. 1990)
Nomilinic acid 4–β-glucoside		$C_{34}H_{46}O_{15}$ M^+ 692.1	*C. limon*	(Matsubara et al. 1990)
Ichangensin		$C_{25}H_{32}O_7$ M^+ 444.4	*Calamondin** *C. limon*	(Manners and Breksa 2004) (Miller et al. 2004)
Methyl isoobacunoate		$C_{27}H_{34}O_8$ M^+ 486.55	*Calamondin**	(Manners and Breksa 2004)
Methyl isoobacunoate diosphenol		$C_{27}H_{32}O_9$ M^+ 502.2	*Calamondin** *C. reticulata*	(Manners and Breksa 2004) (Bennett and Hasegawa 1981)

Table 15.1 contd....

...Table 15.1 contd.

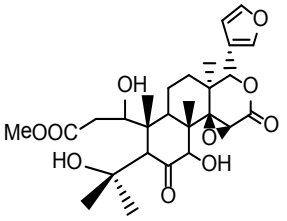
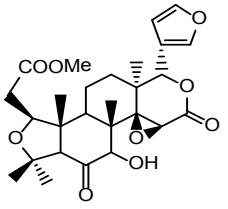
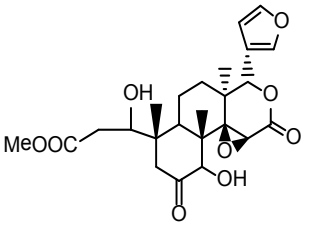
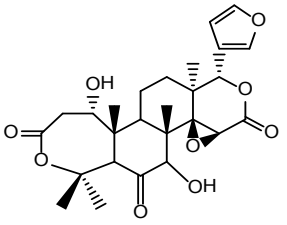
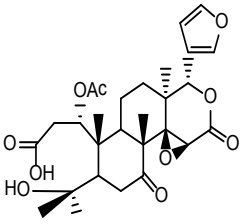
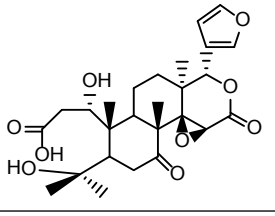
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Calamin		$C_{27}H_{36}O_{10}$ M^+ 520.57	*Calamondin** *C. reticulata*	(Manners and Breksa 2004) (Bennett and Hasegawa 1981)
Cyclocalamin		$C_{27}H_{34}O_9$ M^+ 502.2	*Calamondin** *C. reticulata*	(Manners and Breksa 2004) (Bennett and Hasegawa 1981)
Retrocalamin		$C_{24}H_{30}O_9$ M^+ 462.3	*Calamondin** *C. reticulata*	(Manners and Breksa 2004) (Bennett and Hasegawa 1981)
6-Keto-7β-deacetylnomilol		$C_{26}H_{32}O_9$ M^+ 488.1	*Calamondin**	(Miyake et al. 1992)
Nomilinic acid		$C_{28}H_{36}O_{10}$ M^+ 532.58	*C. reticulata* *C. limon* *Calamondin**	(Ozaki et al. 1991)
Deacetyl nomilinic acid		$C_{26}H_{34}O_9$ M^+ 490.54	*C. reticulata* *C. limon*	(Ozaki et al. 1991)

Table 15.1 contd....

...Table 15.1 contd.

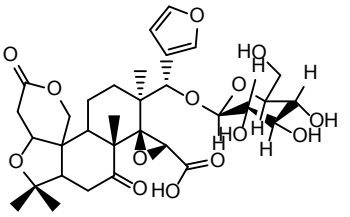
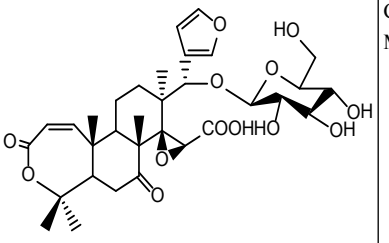
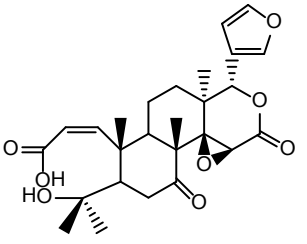
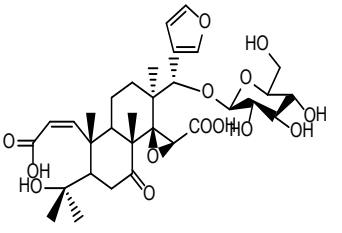
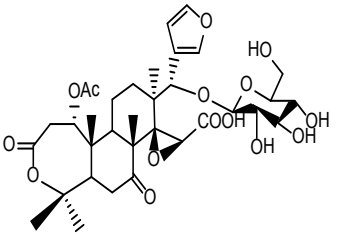
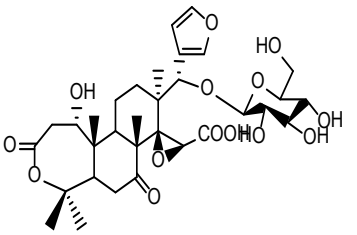
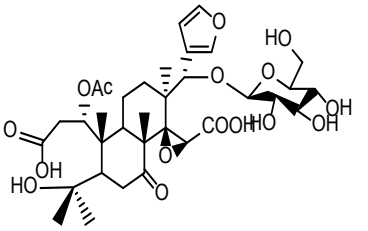
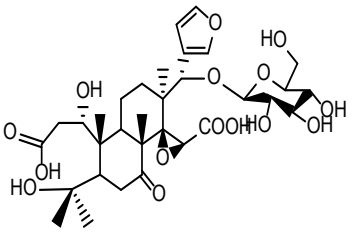
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Limonin glucoside		$C_{32}H_{42}O_{14}$ M^+ 650.67	*C. reticulata* *C. limon*	(Ozaki et al. 1991)
Obacunone glucoside		$C_{32}H_{42}O_{13}$ M^+ 634.67	*C. reticulata* *C. limon*	(Ozaki et al. 1991)
Obacunoic acid		$C_{26}H_{32}O_8$ M^+ 472.53	*C. limon*	(Herman and Hasegawa 1985)
Obacunoic acid glucoside		$C_{32}H_{44}O_{14}$ M^+ 652.53	*C. limon*	(Tian and Schwartz 2003)
Nomilin glucoside		$C_{34}H_{46}O_{15}$ M^+ 694.72	*C. reticulata* *C. limon*	(Ozaki et al. 1991)

Table 15.1 contd....

...Table 15.1 contd.

Compound	Structure	Formula, molecular weight	Source	Reference
Deacetyl nomilin glucoside		$C_{32}H_{44}O_{14}$ M^+ 652.68	*C. reticulata* *C. limon*	(Ozaki et al. 1991)
Nomilinic acid glucoside		$C_{34}H_{48}O_{16}$ M^+ 712.74	*C. reticulata* *C. limon* *Calamondin**	(Ozaki et al. 1991)
Deacetyl nomilinic acid glucoside		$C_{32}H_{46}O_{15}$ M^+ 670.70	*C. reticulata* *C. limon*	(Ozaki et al. 1991)

glucose concentration perfectly alleviates the oxidative damage triggered by hyperglycemia and thus could offer a good candidate for the prohibition of the visual impairment sparked by diabetic retinopathy (Liu et al. 2017a). Similarly, hesperidin may protect retinal ganglial cells from elevated glucose-induced damage owing to its antioxidant properties and blockage of mitochondria-mediated apoptosis (Liu et al. 2017b). Additionally, Apigenin is capable of diminishing glucose uptake by the cancer cells, preventing remodeling of the extracellular matrix as well as the molecules that cause cell adhesion that in turn participate in the dissemination of cancer in addition to prohibiting the development of blood vessels required for tumor growths (Kowalczyk et al. 2017).

The flavonoids contain several phenolic groups, which dissociate under physiological conditions into negatively charged phenolate ions. These phenolate ions bind with the positively charged amino groups in proteins to form a stable ionic bond. Therefore proteins would become fixed and would lose their structural flexibility. Also, phenylpropanoids are characterized by allylic side chain. They are lipophilic, uncharged molecules and can cross biomembranes by free diffusion. Higher concentration of this metabolite can influence membrane fluidity and disturb the interaction of membrane proteins with membrane lipids (Wink 1999, Wink 2008). Examples of some flavonoids and phenyl propanoids isolated from some members of genus *Citrus* is represented in Table 15.2.

Table 15.2 Examples of some flavonoids and phenylpropanoids isolated from some members of genus *Citrus.*

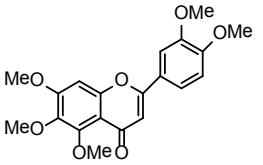
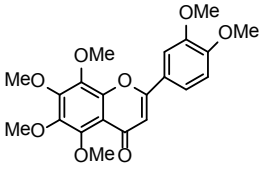
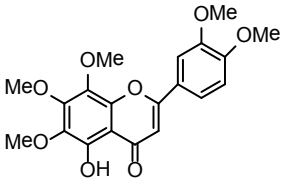
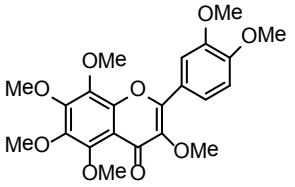
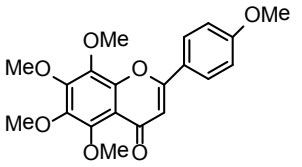
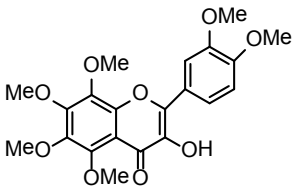
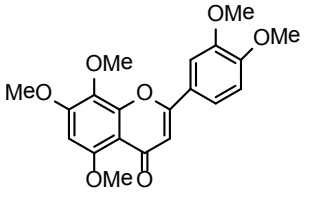
Compound	Structure	Formula, molecular weight	Source	Reference
Polymethoxylated flavones- Sinensetin		$C_{20}H_{20}O_7$ M^+ 372.37	*C. limon* *C. reticulata*	(Robards et al. 1997)
Nobiletin		$C_{21}H_{22}O_8$ M^+ 402.39	*C. limon* *C. reticulata*	(Robards et al. 1997)
5-Demethylnobiletin		$C_{20}H_{20}O_8$ M^+ 388.37	*C. limon* *C. reticulata*	(Kawaii et al. 2000)
Heptamethoxyflavone		$C_{22}H_{24}O_9$ M^+ 432.21	*C. limon* *C. reticulata*	(Robards et al. 1997)
Tangeretin		$C_{20}H_{20}O_7$ M^+ 372.37	*C. limon* *C. reticulata*	(Robards et al. 1997)
Natsudaidain		$C_{21}H_{22}O_9$ M^+ 418.39	*C. limon*	(Kawaii et al. 1999)
Isosinensetin		$C_{20}H_{20}O_7$ M^+ 372.37	*C. limon* *C. reticulata*	(Robards et al. 1997)

Table 15.2 contd....

...Table 15.2 contd.

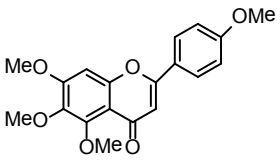
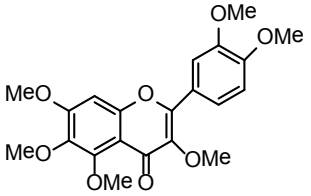
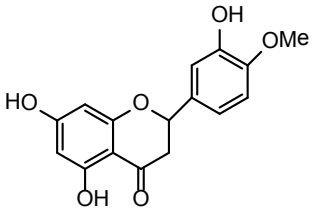
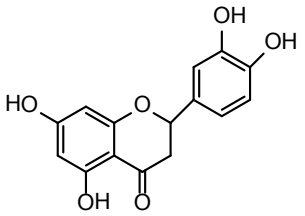
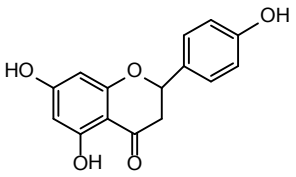
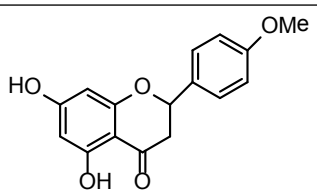
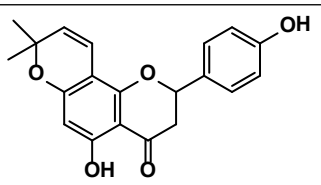
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Tetramethylscutellarein		$C_{19}H_{18}O_6$ M^+ 342.34	*C. limon* *C. reticulata*	(Robards et al. 1997)
Quercetogenin hexamethyl ether		$C_{21}H_{22}O_8$ M^+ 402.39	*C. limon* *C. reticulata*	(Robards et al. 1997)
Flavonoid Aglycones-A-Flavanone Hesperitin		$C_{16}H_{14}O_6$ M^+ 302.28	*C. limon* *C. reticulata*	(Robards et al. 1997)
Eriodictyol		$C_{15}H_{12}O_6$ M^+ 288.25	*C. limon* *C. reticulata*	(Robards et al. 1997)
Naringenin		$C_{15}H_{12}O_5$ M^+ 272.25	*C. limon* *C. reticulata*	(Robards et al. 1997)
Isosakuranetin		$C_{16}H_{14}O_5$ M^+ 286.28	*C. limon* *C. reticulata*	(Robards et al. 1997)
Citflavanone		$C_{20}H_{18}O_5$ M^+ 338.35	*C. medica*	(ITO et al. 1988)

Table 15.2 contd....

...Table 15.2 contd.

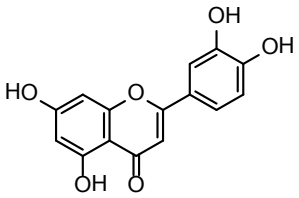
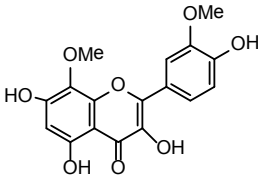
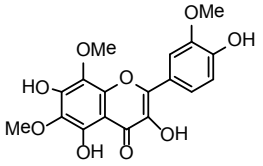
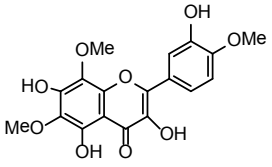
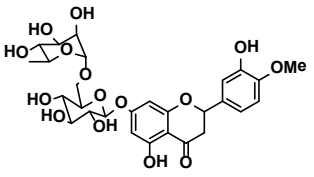
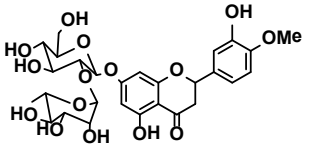
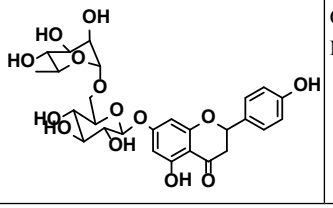
Compound	Structure	Formula, molecular weight	Source	Reference
B-Flavone Luteolin		$C_{15}H_{10}O_6$ M^+ 286.24	*C. limon*	(Kawaii et al. 1999)
Flavonols- 1-Limocitrin		$C_{17}H_{14}O_8$ M^+ 346.29	*C. limon*	(Baldi et al. 1995)
2-Limocitrol		$C_{18}H_{16}O_9$ M^+ 376.31	*C. limon*	(Baldi et al. 1995)
Isolimocitrol		$C_{18}H_{16}O_9$ M^+ 376.31	*C. limon*	(Baldi et al. 1995)
Flavonoid glycosides- **A-Flavanone glycosides-** Hesperidin		$C_{28}H_{34}O_{15}$ M^+ 610.56	*C. limon* *C. reticulata*	(Robards et al. 1997)
Neohesperidin		$C_{28}H_{34}O_{15}$ M^+ 610.56	*C. limon* *C. reticulata*	(Bocco et al. 1998)
Narirutin		$C_{27}H_{32}O_{14}$ M^+ 580.53	*C. limon* *C. reticulata*	(Robards et al. 1997) (Bocco et al. 1998)

Table 15.2 contd....

...Table 15.2 contd.

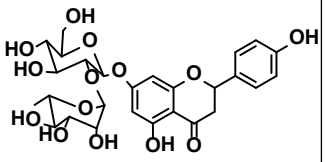
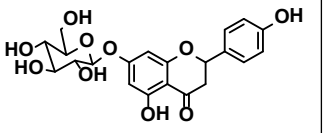
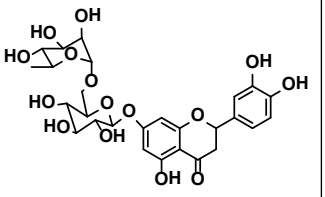
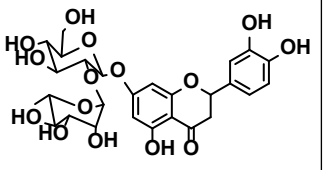
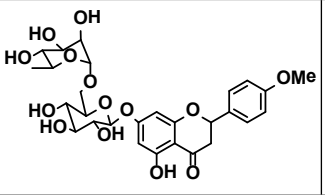
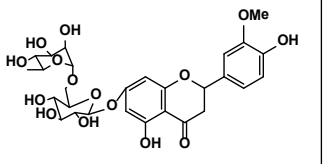
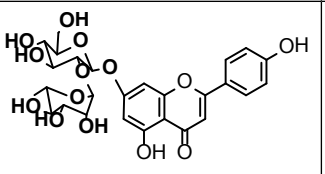
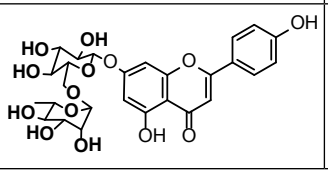
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Naringin		$C_{27}H_{32}O_{14}$ M^+ 580.53	*C. limon* *C. reticulata*	(Bocco et al. 1998)
Naringenin7-O-glucoside		$C_{21}H_{22}O_{10}$ M^+ 434.39	*C. limon*	(Baldi et al. 1995)
Eriocitrin		$C_{27}H_{32}O_{15}$ M^+ 596.53	*C. limon* *C. reticulata*	(Robards et al. 1997) (Bocco et al. 1998)
Neoeriocitrin		$C_{27}H_{32}O_{15}$ M^+ 596.53	*C. limon* *C. reticulata*	(Bocco et al. 1998)
Didymin (neoponcirin)		$C_{28}H_{34}O_{14}$ M^+ 594.56	*C. limon* *C. reticulata*	(Kawaii et al. 1999) (Robards et al. 1997)
Homoeriodictyol 7-O-rutinoside		$C_{28}H_{34}O_{15}$ M^+ 610.53	*C. limon*	(Gil-Izquierdo et al. 2004)
B- Flavone glycosides- Rhoifolin		$C_{27}H_{30}O_{14}$ M^+ 578.52	*C. limon*	(Nogata et al. 2006)
Isorhoifolin		$C_{27}H_{30}O_{14}$ M^+ 578.52	*C. limon* *C. reticulata*	(Kawaii et al. 1999)

Table 15.2 contd....

...Table 15.2 contd.

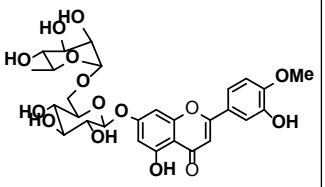
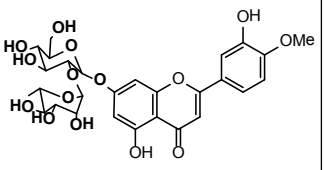
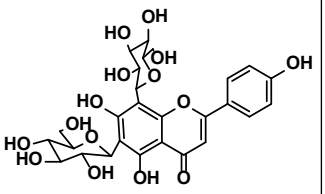
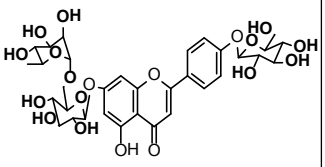
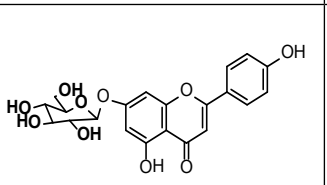
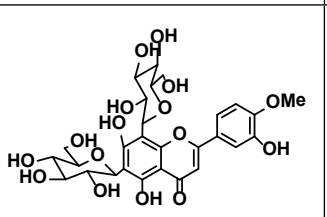
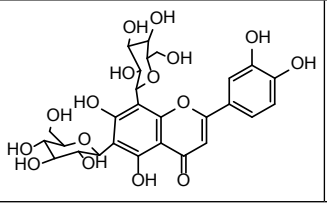
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Diosmin		$C_{28}H_{32}O_{15}$ M^+ 608.54	*C. limon* *C. reticulata*	(Kawaii et al. 1999)
Neodiosmin		$C_{28}H_{32}O_{15}$ M^+ 608.54	*C. reticulata*	(Nogata et al. 2006)
Vicenin		$C_{27}H_{30}O_{15}$ M^+ 594.52	*C. limon*	(Tusa et al. 2007)
Narirutin 4′-glucoside		$C_{33}H_{42}O_{19}$ M^+ 842.1	*C. limon* *C. reticulata*	(Manthey and Grohmann 2001)
Apigenin 7-glucoside		$C_{21}H_{20}O_{10}$ M^+ 432.38	*C. limon*	(Dugo et al. 2005)
Diosmetin 6,8-di-C-glucoside		$C_{28}H_{32}O_{16}$ M^+ 624.54	*C. limon* *C. reticulata*	(Dugo et al. 2005)
Luteolin 6,8-di-C-glucoside		$C_{27}H_{30}O_{16}$ M^+ 610.52	*C. limon*	(Baldi et al. 1995)

Table 15.2 contd....

...Table 15.2 contd.

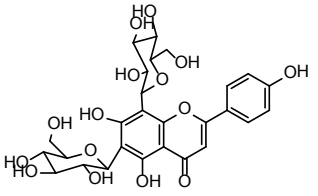
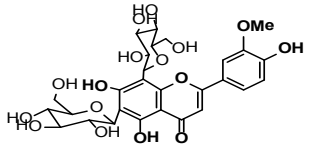
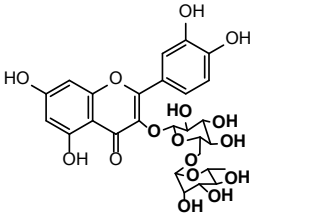
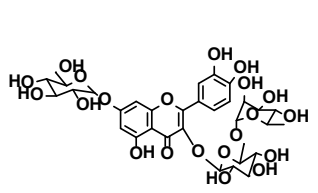
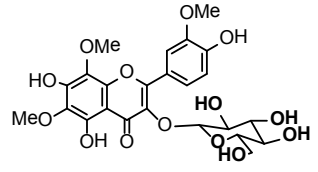
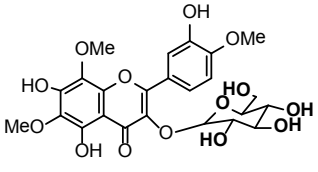
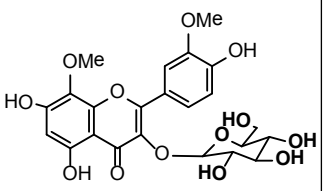
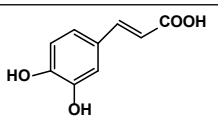
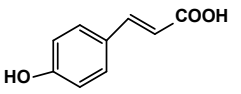
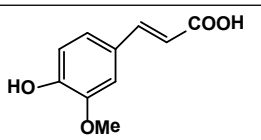
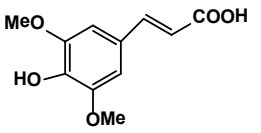
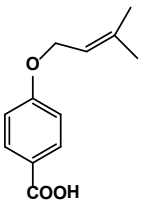
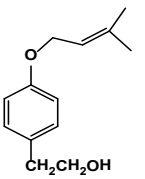
Compound	**Structure**	**Formula, molecular weight**	**Source**	**Reference**
Apigenin 6,8-di-C-glucoside		$C_{27}H_{30}O_{15}$ M^+ 594.52	*C. limon* *C. reticulata*	(Dugo et al. 2005)
Chrysoeriol 6,8-di-C-glucoside		$C_{28}H_{32}O_{16}$ M^+ 624.54	*C. limon*	(Gil-Izquierdo et al. 2004)
C-Flavonol glycosides- Rutin		$C_{27}H_{30}O_{16}$ M^+ 610.52	*C. limon* *C. reticulata*	(Baldi et al. 1995) (Nogata et al. 2006)
Quercetin-3-O rutinoside-7-O glucoside		$C_{33}H_{40}O_{21}$ M^+ 772.66	*C. limon*	(Gil-Izquierdo et al. 2004)
Limocitrol 3-β D-glucoside		$C_{24}H_{26}O_{14}$ M^+ 538.45	*C. limon*	(Dugo et al. 2005)
Isolimocitrol 3-β D-glucoside		$C_{24}H_{26}O_{14}$ M^+ 538.45	*C. limon*	(Dugo et al. 2005)

Table 15.2 contd....

...Table 15.2 contd.

Compound	Structure	Formula, molecular weight	Source	Reference
Limocitrin 3-β D-glucoside		$C_{23}H_{24}O_{13}$ M^+ 508.43	*C. limon*	(Dugo et al. 2005)
Phenolic acids- Caffeic acid		$C_9H_8O_4$ M^+ 180.16	*C. limon* *C. reticulata*	(Bocco et al. 1998)
P-coumaric acid		$C_9H_8O_3$ M^+ 164.16	*C. limon* *C. reticulata*	(Bocco et al. 1998)
Ferulic acid		$C_{10}H_{10}O_4$ M^+ 194.18	*C. limon* *C. reticulata*	(Bocco et al. 1998)
Sinapic acid		$C_{11}H_{12}O_5$ M^+ 224.21	*C. limon* *C. reticulata*	(Bocco et al. 1998)
Valencic acid		$C_{12}H_{14}O_3$ M^+ 206.24	*C. medica*	(ITO et al. 1988)
Phenolic alcohol- Etrogol		$C_{13}H_{18}O_2$ M^+ 206.24	*C. medica*	(ITO et al. 1988)

Biological activity of coumarins

The genus *Citrus* constitutes one of the highly popular genera that are very rich in oxyprenylated coumarins (Genovese et al. 2018). Most of the isolated coumarins from genus *Citrus* plants were found to possess antioxidant (Yu et al. 2005), antibacterial (Damu et al. 2005), antifungal (Ju-Ichi et al. 1988), cardiovascular (Takeuti et al. 1991), antiplatelet (Teng et al. 1992), and antitumor effects (Berhow et al. 1994). Nordentatin revealed a promising antibacterial activity against many microbes such as *Bacillus subtilis, Bordetella brochiseptica, Pseudomonas aeruginosa, Staphylococcus aureus,* and *S. lutea* (Wu and Furukawa 1982).

Moreover, seselin and xanthyletin increased heart rate and slightly decreased blood pressure, moreover seselin showed calcium blocking activity (Takeuti et al. 1991). Other biological activities such as anticancer, antiproliferative, anti-inflammatory were also reported for some isolated coumarins (Gunatilaka et al.

1994, Garcia-Argaez et al. 2000, Kawaii et al. 2001, Murakami et al. 2005). Coumarins contain phenolic hydroxyl group which dissociate into negatively charged ions. This ion binds to positively charged amino acid residue in protein, the proteins would be become fixed and that might lead to loss of function. Moreover, furanocoumarins are intercalating secondary metabolites (Wink 2008). Some coumarins isolated from a few members of genus *Citrus* representatives are represented in Table 15.3.

Biological activity of sterols

The plant sterols are known to reduce the total and Low-Density Lipoprotein (LDL) (Normen et al. 1999) and a high dietary intake might therefore improve health. Several investigators stated those sterols as *β*-sitosterol, campesterol, and stigmasterol exhibited numerous biological effects, e.g., hypocholestermic, anticancer activity against colon, breast and prostate malignant tumors, immunostimulant activity, antibacterial and antifungal effects (Potter 1995, Awad and Fink 2000, Matvienko et al. 2002, Ju et al. 2004).

Sterols are lipophilic secondary metabolites, which can intercalate between DNA bases that lead to a stabilization of the DNA double helix. This stabilization disturbs the activity of DNA polymerase and this leads to frame shift mutations in the corresponding protein. In addition, sterols are lipophilic and uncharged molecules that can cross biomembranes by free diffusion. Therefore, at higher concentrations, sterol can influence membrane fluidity and disturb the interaction of membrane proteins with membrane lipids (Wink 2008). Most of the relevant isolated sterols from some members of genus *Citrus* are found in Table 15.4.

Biological activity of acridone alkaloids

Several authors reported the acridone alkaloids isolated from *Citrus* species exhibited antiviral, antimicrobial, antifungal, mutagenic, antineoplastic, anti-herpes and antimalarial effects (Wolters and Eilert 1981, Yang et al. 1987, Paulini et al. 1991). Acridone alkaloids are reported to have mutagenic and carcinogenic properties through intercalation of DNA and related target of the organism (Wink 2008). Examples of some acridone alkaloids isolated from some members of genus *Citrus* is represented in Table 15.5.

Biological activity of essential oils

Citrus essential oils are a major source for many natural flavoring materials of commercial importance. They are widely used in beverages, candy, frozen, desserts, confectionary, pharmaceuticals, cosmetics and perfumery industry. As a result, extensive research efforts have been directed towards analysis of these oils. Limonene was the main constituent in mandarin peel oils (67.0%) while γ-terpinene (53.0%) and linalool (16.1%) were the chief constituents of leaf oil (Njoroge et al. 2005, Karioti et al. 2007).

Singh et al. (1999) analyzed the chemical constituents of leaf and peel essential oil of *C. medica* by HPLC, GLC and GLC-MS. Twenty four components (99.7%) of the leaf oil were identified and the main constituents were citronellal (63.3%), citronellol (15.1%), limonene (8.0%), citronellyl acetate (5.2%), isopulegol (1.6%), and linalool (1.3%). While thirty five components of the peel oil, which amounting 99.1% of the total identified components, and the major constituents are limonene (32.0%), citronellal (27.5%), citronellol (13.0%), p-cymene (6.5%), geranial (2.3%), γ-terpinene (2.0%), citronellic acid (1.8%), α-terpineol (1.2%), and linalool (1.2%) (Lota et al. 1999).

The leaf and peel essential oil of *C. medica* was studied and their components were investigated. Three chemotypes- limonene, limonene/γ-terpinene and limonene/geranial/neral were observed for the peel oil while leaf oil exhibited the limonene/geranial/neral composition. On the other hand, the peel and leaf oils of *C. limon* L. were analyzed where 42 and 27 components were identified. The main constituents of the lemon peel were limonene (70.4%), while the leaf oil consisted mainly of limonene (40.8%), and β-pinene (18.5%) (Singh et al. 1999).

A comparative study between the volatile components of *C. junos* with those of *C. limon* have been studied. The two oils were distinctively different. In the *C. junos* oil, alcohols were the second major components (2.05%), while in the *C. limon* aldehydes (2.10%) and esters (0.63%) were more dominant (Njoroge et al. 1994).

Table 15.3 Examples of some coumarins isolated from some members of genus *Citrus*.

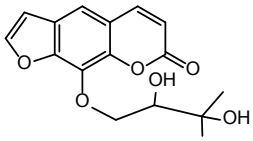
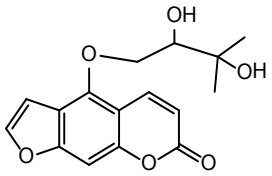
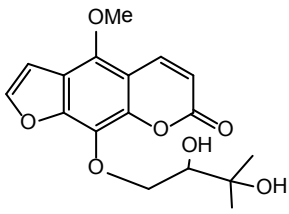
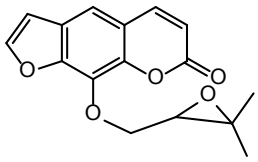
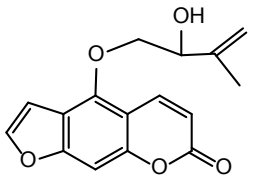
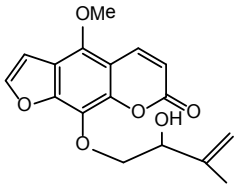
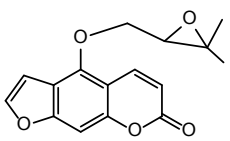
Compound	**Structure**	**Formula, molecular weight**	**Source**	**References**
Heraclenol		$C_{16}H_{16}O_6$ M^+ 304.29	*C. limon* oil	(Ziegler and Spiteller 1992)
Oxypeucedanin hydrate		$C_{16}H_{16}O_6$ M^+ 304.29	*C. limon* oil	(Ziegler and Spiteller 1992)
Byakangelicin		$C_{17}H_{18}O_7$ M^+ 334.32	*C. limon* oil	(Ziegler and Spiteller 1992)
Heraclenin		$C_{16}H_{14}O_5$ M^+ 286.28	*C. limon* oil	(Ziegler and Spiteller 1992)
Pabulenol		$C_{16}H_{14}O_5$ M^+ 286.28	*C. limon* oil	(Ziegler and Spiteller 1992)
Neobyakangelicol		$C_{17}H_{16}O_6$ M^+ 316.31	*C. limon* oil	(Ziegler and Spiteller 1992)
Oxypeucedanin		$C_{16}H_{14}O_5$ M^+ 286.28	*C. limon* oil	(Ziegler and Spiteller 1992)

Table 15.3 contd....

...Table 15.3 contd.

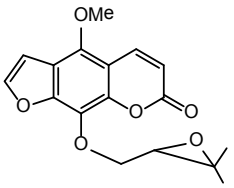
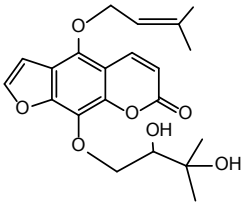
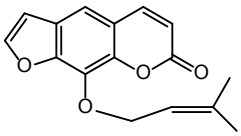
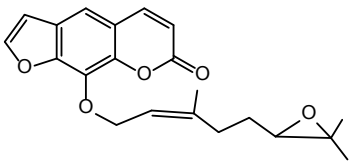
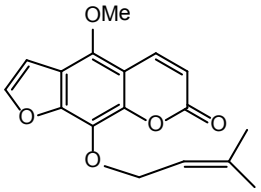
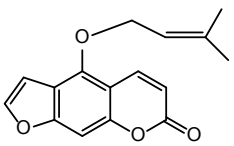
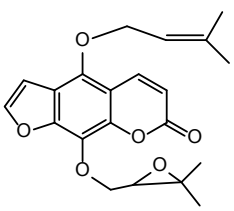
Compound	**Structure**	**Formula, molecular weight**	**Source**	**References**
Byakangelicol		$C_{17}H_{16}O_6$ M^+ 316.31	*C. limon* oil	(Ziegler and Spiteller 1992)
5-(3'-Methylbut-2'-enyloxy)-8-(2',3'-dihydroxy-3'-methylbutyloxy) psoralen		$C_{21}H_{24}O_7$ M^+ 388.29	*C. limon* oil	(Ziegler and Spiteller 1992)
Imperatorin		$C_{16}H_{14}O_4$ M^+ 270.28	*C. limon* oil	(Ziegler and Spiteller 1992)
8-(7',8'-Epoxygeranyloxy) psoralin		$C_{21}H_{22}O_5$ M^+ 354.12	*C. limon* oil	(Ziegler and Spiteller 1992)
Phellopterin		$C_{17}H_{16}O_{56}$ M^+ 300.31	*C. limon* oil, leaves	(Ziegler and Spiteller 1992)
Isoimperatorin		$C_{16}H_{14}O_4$ M^+ 270.28	*C. limon* oil	(Ziegler and Spiteller 1992)
5-(3'-Methylbut-2'-enyloxy)-8-(2',3'-epoxy3'-methylbutyloxy)psoralen		$C_{21}H_{22}O_6$ M^+ 370.12	*C. limon* oil	(Ziegler and Spiteller 1992)

Table 15.3 contd....

...Table 15.3 contd.

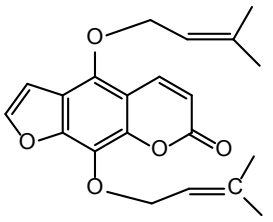
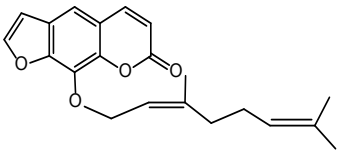
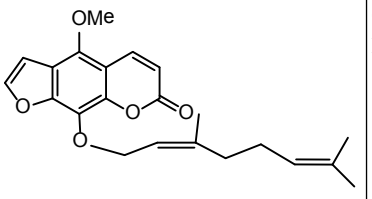
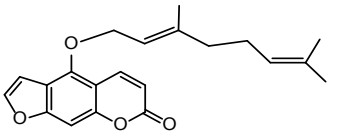
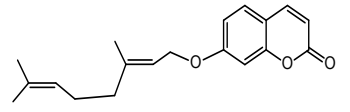
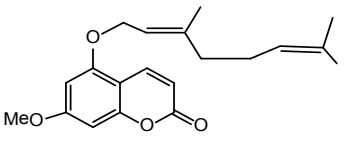
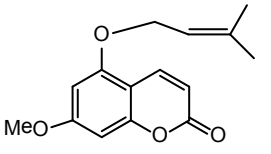
Compound	Structure	Formula, molecular weight	Source	References
Cnidicin		$C_{21}H_{22}O_5$ M^+ 354.40	*C. limon* oil	(Ziegler and Spiteller 1992)
8-Geranyloxypsoralen		$C_{21}H_{22}O_4$ M^+ 338.40	*C. limon* oil	(Ziegler and Spiteller 1992)
5-Methoxy-8-geranyloxypsoralen		$C_{22}H_{24}O_5$ M^+ 368.40	*C. limon* oil	(Ziegler and Spiteller 1992)
Bergamottin		$C_{21}H_{22}O_4$ M^+ 338.40	*C. limon* oil	(Ziegler and Spiteller 1992)
Aurapten		$C_{19}H_{22}O_3$ M^+ 298.38	*C. limon* oil	(Ziegler and Spiteller 1992)
5-Geranyloxy-7-methoxycoumarin		$C_{20}H_{24}O_4$ M^+ 328.40	*C. limon* oil	(Ziegler and Spiteller 1992)
5-(3'-Methylbut-2'-enyloxy)-7-methoxycoumarin		$C_{15}H_{16}O_4$ M^+ 260.29	*C. limon* oil	(Ziegler and Spiteller 1992)

Table 15.3 contd....

...Table 15.3 contd.

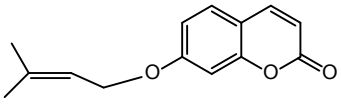
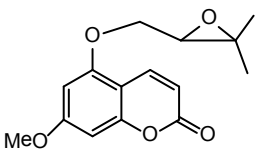
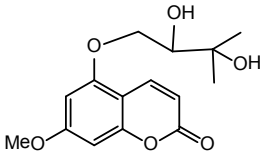
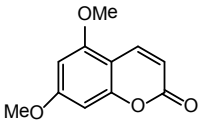
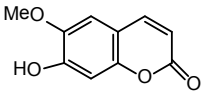
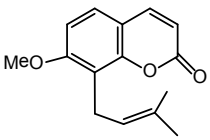
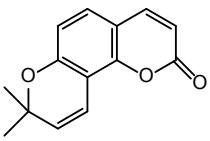
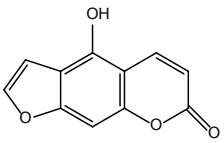
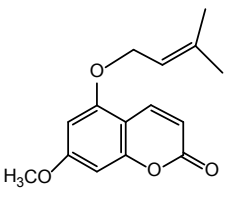
Compound	**Structure**	**Formula, molecular weight**	**Source**	**References**
7-(3'-Methylbut-2'-enyloxy) coumarin		$C_{14}H_{14}O_3$ M^+ 230.29	*C. limon* oil	(Ziegler and Spiteller 1992)
5-(2',3'-Epoxy-3'-methylbutyloxy)-7-methoxycoumarin		$C_{15}H_{16}O_5$ M^+ 276.29	*C. limon* oil	(Ziegler and Spiteller 1992)
5-(2',3'-Dihydroxy-3'-methylbutyloxy)-7-methoxycoumarin		$C_{15}H_{18}O_6$ M^+ 294.21	*C. limon* oil	(Ziegler and Spiteller 1992)
Citropten		$C_{11}H_{10}O_4$ M^+ 206.19	*C. limon* oil *C. reticulata*	(Nordby and Nagy 1981, Ziegler and Spiteller 1992)
Scopoletin		$C_{10}H_8O_4$ M^+ 192.17	*C. limon*	(Zobel et al. 1991)
Osthol		$C_{15}H_{16}O_3$ M^+ 244.29	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Seselin		$C_{14}H_{12}O_3$ M^+ 228.24	*C. limon* *C. reticulata*	(Zobel et al. 1991) (Nordby and Nagy 1981)
Bergaptol		$C_{11}H_6O_4$ M^+ 202.16	*C. limon* oil	(Ziegler and Spiteller 1992)
Limettin a		$C_{15}H_{16}O_4$ M^+ 260.12	*C. limon* oil	(Ziegler and Spiteller 1992)

Table 15.3 contd....

...Table 15.3 contd.

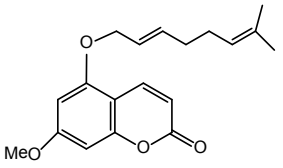
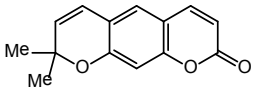
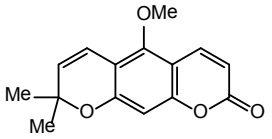
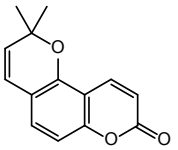
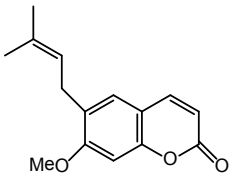
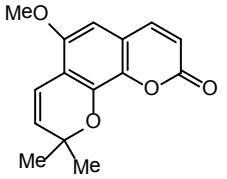
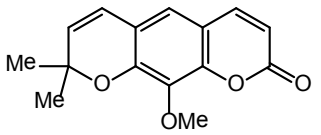
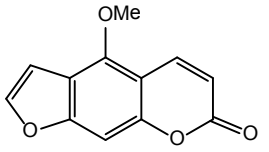
Compound	Structure	Formula, molecular weight	Source	References
Limettin b		$C_{20}H_{24}O_4$ M^+ 330.12	*C. limon* oil	(Ziegler and Spiteller 1992)
Xanthyletin		$C_{14}H_{12}O_3$ M^+ 228.24	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Xanthoxyletin		$C_{15}H_{14}O_4$ M^+ 258.27	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Alloxanthoxyletin		$C_{15}H_{14}O_4$ M^+ 258.27	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Suberosin		$C_{15}H_{16}O_3$ M^+ 244.29	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Braylin		$C_{15}H_{14}O_4$ M^+ 258.27	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Luvangetin		$C_{15}H_{14}O_4$ M^+ 258.27	*C. limon* *C. reticulata*	(Nordby and Nagy 1981)
Bergapten		$C_{12}H_8O_4$ M^+ 216.19	*C. limon* oil	(Chouchi and Barth 1994)

Table 15.3 contd....

...Table 15.3 contd.

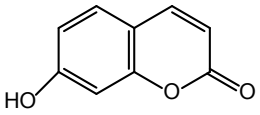
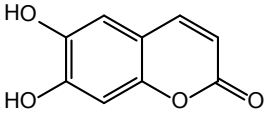
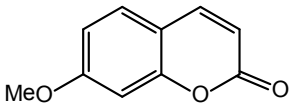
Compound	Structure	Formula, molecular weight	Source	References
Umbelliferone		$C_9H_6O_3$ M^+ 162.14	*C. limon* juice	(Feldman and Hanks 1965)
Esculetin		$C_9H_6O_4$ M^+ 178.14	*C. limon* root, leaves	(Feldman and Hanks 1965)
Herniarin		$C_{10}H_8O_3$ M^+ 176.17	*C. limon* oil	(Chouchi and Barth 1994)

Table 15.4 Examples of some sterols isolated from some members of genus *Citrus*.

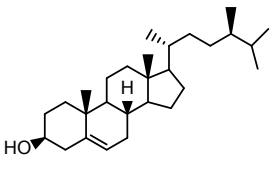
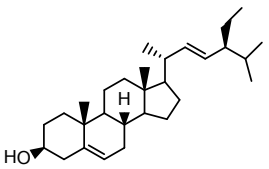
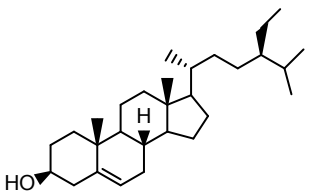
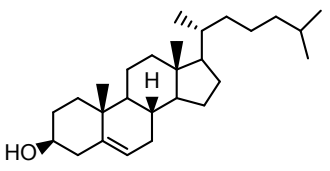
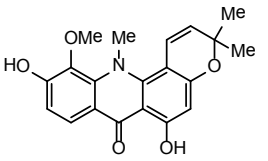
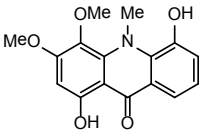
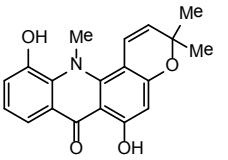
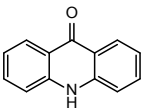
Compound	Structure	Formula, M. Wt	Source	References
Campesterol		$C_{28}H_{48}O$ M^+ 400.69	*C. limon* *C. reticulata*	(Jiménez-Escrig et al. 2006) (Douglas and Sykes 1985)
Stigmasterol		$C_{29}H_{48}O$ M^+ 412.69	*C. limon* *C. reticulata*	(Jiménez-Escrig et al. 2006) (Douglas and Sykes 1985)
β-Sitosterol		$C_{29}H_{50}O$ M^+ 414.71	*C. limon* *C. reticulata*	(Jiménez-Escrig et al. 2006) (Douglas and Sykes 1985)
Cholesterol		$C_{27}H_{46}O$ M^+ 386.66	*C. reticulata*	(Douglas and Sykes 1985)

Table 15.5 Examples of some acridone alkaloid isolated from some members of genus *Citrus*.

Compound	Structure	Formula, molecular weight	Source	References
Citracridone I		$C_{20}H_{19}NO_5$ M^+ 353	*C. limon*	(Chang 1990)
Citrusinine I		$C_{16}H_{15}NO_5$ M^+ 301	*C. limon*	(Chang 1990)
5-Hydroxynoracronycine		$C_{19}H_{17}NO_4$ M^+ 323	*C. limon*	(Chang 1990)
9(10H)-Acridinone		$C_{13}H_9NO$ M^+ 195	*C. reticulata*	(Sun et al. 2007)

It was reported that *C. reticulata* and *C. medica* essential oils were found to exhibit a promising fungitoxic activity with growth inhibition of 45% and 30%, respectively. *C. limon* and *C. medica* essential oils were screened on both MCF-7 and HeLa. IC_{50} for HeLa cell line was 17 µg/mL and 1 µg/mL for *C. limon* and *C. medica*, respectively. In addition, they found that *C. limon* had a greater cytotoxic effect on MCF-7 than that on HeLa cells (Tripathi et al. 2008, Monajemi et al. 2010).

The antifungal efficacy, antiviral and the higher significant reduction of DPPH radicals (antioxidant) of the mandarin oils and the activity might be related to the presence of N-methylanthranilate (Schnitzler et al. 2008). Recently, the essential oil of *Citrus lumia* Risso had shown a pronounced antioxidant, anti-cholinesterase, and neuroactive with a potent acetylcholinesterase inhibitory activity as well as anti-cholinesterase using both cell-free in addition to cell-based assays (Smeriglio et al. 2017). Moreover, the essential oil of *Citrus aurantium* L. presented a potent anxiolytic activity in cocaine users who were subjected to Simulated Public Speaking (Chaves Neto et al. 2017).

Essential oil contains lipophilic terpenoids which can enter into the membrane of the cell and form hydrophobic interaction with the lipophilic side chains of phospholipids or cholesterol. Higher concentrations can influence membrane fluidity and disturb the interaction of membrane proteins with the membrane lipids, which are important for proteins correct three dimensional conformation. Any change in protein will modulate its activity (Wink 2008).

Conclusions

The family Rutaceae in general and genus *Citrus* in particular act as a natural everlasting source of medicinally effective phytoconstituents that could be of high relevance in curing a wide array of ailments. Undoubtedly this is attributed to their richness by several classes of active constituents comprising limonoids, flavonoids, coumarins and essential oils. Nevertheless, more thorough phytochemical and biological studies should be done on many of its species to discover more of its biological importance and secondary metabolites guided by its earlier traditional popularity.

References

Andrews, A.C. 1961. Acclimatization of Citrus fruits in the Mediterranean region. Agr. Hist. 35: 35–46.

Arias, B.A. and Ramón-Laca, L. 2005. Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. J. Ethnopharmacol. 97: 89–95.

Awad, A.B. and Fink, C.S. 2000. Phytosterols as anticancer dietary components: evidence and mechanism of action. J. Nutr. 130: 2127–2130.

Baldi, A., Rosen, R.T., Fukuda, E.K. and Ho, C.-T. 1995. Identification of nonvolatile components in lemon peel by high-performance liquid chromatography with confirmation by mass spectrometry and diode-array detection. J. Chromatogr. A. 718: 89–97.

Bennett, R. and Hasegawa, S. 1981. Limonoids of calamondin seeds. Tetrahedron. 37: 17–24.

Berhow, M.A., Bennett, R.D., Poling, S.M., Vannier, S., Hidaka, T. and Omura, M. 1994. Acylated flavonoids in callus cultures of *Citrus aurantifolia*. Phytochemistry 36: 1225–1227.

Berhow, M.A., Hasegawa, S. and Manners, G.D. 2000. *Citrus* Limonoids: Functional Chemicals in Agriculture and Food, ACS Publications.

Bocco, A., Cuvelier, M.-E., Richard, H. and Berset, C. 1998. Antioxidant activity and phenolic composition of citrus peel and seed extracts. J. Agr. Food Chem. 46: 2123–2129.

Bracke, M., Bruyneel, E., Vermeulen, S.J., Vennekens, K.l., Vanmarck, V. and Mareel, M. 1994. Citrus flavonoid effect on tumor invasion and metastasis. Food Technol. 48: 121–124.

Breksa, A.P. and Ibarra, P. 2007. Colorimetric method for the estimation of total limonoid aglycones and glucoside contents in Citrus juices. J. Agr. Food Chem. 55: 5013–5017.

Breksa, A.P., Zukas, A.A. and Manners, G.D. 2005. Determination of limonoate and nomilinoate A-ring lactones in Citrus juices by liquid chromatography–electrospray ionization mass spectrometry. J. Chromatograph. A 1064: 187–191.

Cai, H., Kharel, G.P. and Hashinaga, F. 1993. Changes in the limonoid content during development of citron fruit (*Citrus medica* L.). J. JPN Soc. Hortic. Sci. 62: 215–220.

Chang, K. 1992. The evaluation of citrus demand and supply. Proceeding of International Society Citric. Italy 3: 1153–1155.

Chang, S.-H. 1990. Flavonoids, coumarins and acridone alkaloids from the root bark of *Citrus limonia*. Phytochemistry 29: 351–353.

Chaves Neto, G., Braga, J.E.F., Alves, M.F., de Morais Pordeus, L.C., Dos Santos, S.G., Scotti, M.T., Almeida, R.N. and Diniz, M.F.F.M. 2017. Anxiolytic effect of *Citrus aurantium* L. in crack users. Evid.-Based Compl. Alt. 2017, doi:10.1155/2017/7217619.

Chouchi, D. and Barth, D. 1994. Rapid identification of some coumarin derivatives in deterpenated citrus peel oil by gas chromatography. J. Chromatogr. A 672: 177–183.

Cook, N. and Samman, S. 1996. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem. 7: 66–76.

Damu, A. G., Kuo, P.-C., Shi, L.-S., Li, C.-Y., Kuoh, C.-S., Wu, P.-L. and Wu, T.-S. 2005. Phenanthroindolizidine alkaloids from the stems of *Ficus septica*. J. Nat. Prod. 68: 1071–1075.

Del Caro, A., Piga, A., Vacca, V. and Agabbio, M. 2004. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chem. 84: 99–105.

Douglas, T. and Sykes, S. 1985. Phospholipid, galactolipid and free sterol composition of fibrous roots from citrus genotypes differing in chloride exclusion ability. Plant. Cell Environ. 8: 693–699.

Dugo, P., Lo Presti, M., Öhman, M., Fazio, A., Dugo, G. and Mondello, L. 2005. Determination of flavonoids in citrus juices by micro-HPLC-ESI/MS. J. Sep. Sci. 28: 1149–1156.

FAO. 2006. Citrus fruit–fresh and processed, annual statistics, 2003. Commodities and Trade Division. FAO of the UN, Rome.

Feldman, A. and Hanks, R. 1965. Phenolic compounds in roots and leaves of four citrus cultivars. Nature. 207: 985–986.

Filatova, L.A.K., Y. . 1999. The significance of flavonoids from citrus juices in disease prevention. Pishchev. Promyshlen. 8: 62–63.

Garcia-Argaez, A.N., Apan, T.O.R., Delgado, H.P., Velázquez, G. and Martínez-Vázquez, M. 2000. Anti-inflammatory activity of coumarins from *Decatropis bicolor* on TPA ear mice model. Planta Med. 66: 279–281.

Genovese, S., Taddeo, V.A, Epifano, F. and Fiorito, S. 2018. Prenylated coumarins of the genus *Citrus*: An overview of the 2006–2016 literature data. Curr. Med. Chem., doi:10.2174/0929867325666180104154443.

Gil-Izquierdo, A., Riquelme, M.T., Porras, I. and Ferreres, F. 2004. Effect of the rootstock and interstock grafted in lemon tree (*Citrus limon* (L.) Burm.) on the flavonoid content of lemon juice. J. Agr. Food Chem. 52: 324–331.

Govindachari, T., Suresh, G., Gopalakrishnan, G., Masilamani, S. and Banumathi, B. 2000. Antifungal activity of some tetranortriterpenoids. Fitoterapia. 71: 317–320.

Gunatilaka, A.L., Kingston, D.G., Wijeratne, E.K., Bandara, B.R., Hofmann, G.A. and Johnson, R.K. 1994. Biological activity of some coumarins from Sri Lankan Rutaceae. J. Nat. Prod. 57: 518–520.

Hasegawa, S., Bennett, R.D. and Herman, Z. 1986. A limonoid from young calamondin seedlings. Phytochemistry 25: 1984–1985.

Herman, Z. and Hasegawa, S. 1985. Limonin biosynthesis from obacunone via obacunoate in Citrus limon. Phytochemistry 24: 2911–2913.

Hume, H.H. 1957. Citrus fruits. *Citrus* fruits. The Macmilian Company, New York, 1957.

Ishiwa, J., Sato, T., Mimaki, Y., Sashida, Y., Yano, M. and Ito, A. 2000. A citrus flavonoid, nobiletin, suppresses production and gene expression of matrix metalloproteinase 9/gelatinase B in rabbit synovial fibroblasts. J. Rheumatol. 27: 20–25.

Ito, C., Mizuno, T., Matsuoka, M.M., Kimura, Y., Sato, K., Kajiura, I., Omura, Mitsuo, Juichi, M. and Furukawa, H. 1988. Constituents of domestic Citrus plants. A new flavonoid and other new components from Citrus plants. Chem. Pharm. Bull. 36: 3292–3295.

Jayaprakasha, G., Singh, R., Pereira, J. and Sakariah, K. 1997. Limonoids from *Citrus reticulata* and their moult inhibiting activity in mosquito Culex quinquefasciatus larvae. Phytochemistry 44: 843–846.

Jiménez-Escrig, A., Santos-Hidalgo, A. B. and Saura-Calixto, F. 2006. Common sources and estimated intake of plant sterols in the Spanish diet. J. Agr. Food Chem. 54: 3462–3471.

Ju-Ichi, M., Inoue, M., Ikegami, M., Kajiura, I., Omura, M. and Furukawa, H. 1988. New coumarins from *Citrus funadoko*. Heterocycles 27: 1451–1454.

Ju, Y.H., Clausen, L.M., Allred, K.F., Almada, A.L. and Helferich, W.G. 2004. β-sitosterol, β-sitosterol glucoside, and a mixture of β-sitosterol and β-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells *in vitro* and in ovariectomized athymic mice. J. Nutr. 134: 1145–1151.

Jung, U., Kim, H., Lee, J., Lee, M., Kim, H., Park, E., Kim, H., Jeong, T. and Choi, M. 2003. Naringin supplementation lowers plasma lipids and enhances erythrocyte antioxidant enzyme activities in hypercholesterolemic subjects. Clin. Nutr. 22: 561–568.

Karioti, A., Skaltsa, H. and Gbolade, A.A. 2007. Constituents of the distilled essential oils of *Citrus reticulata* and *C. paradisi* from Nigeria. J. Essent. Oil Res. 19: 520–522.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K. and Yano, M. 1999. HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from Citrus juices. J. Agr. Food Chem. 47: 128–135.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M., Koizumi, M., Ito, C. and Furukawa, H. 2000. Quantitative study of flavonoids in leaves of Citrus plants. J. Agr. Food Chem. 48: 3865–3871.

Kawaii, S., Tomono, Y., Ogawa, K., Sugiura, M., Yano, M., Yoshizawa, Y., Ito, C. and Furukawa, H. 2001. Antiproliferative effect of isopentenylated coumarins on several cancer cell lines. Anticancer Res. 21: 1905–1911.

Khalil, A.T., Maatooq, G.T. and El Sayed, K.A. 2003. Limonoids from *Citrus reticulata*. Z. Naturforsch. C. 58: 165–170.

Kim, H.K., Jeong, T.-S., Lee, M.-K., Park, Y.B. and Choi, M.-S. 2003. Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. Clin. Chim. Acta. 327: 129–137.

Kowalczyk, A., Bodalska, A., Miranowicz, M. and Karłowicz-Bodalska, K. 2017. Insights into novel anticancer applications for apigenin. Adv. Clin. Exp. Med. 26: 1143–1146.

Ladaniya, M. 2008. Citrus fruit: biology, technology and evaluation San Diego: Academic.(p. 198).

Lam, L. K. and Hasegawa, S. 1989. Inhibition of Benzo [a] pyrene-Induced Forestomach Neoplasia in Mice by Citrus Limonoids.

Lee, L.-T., Huang, Y.-T., Hwang, J.-J., Lee, A. Y.-L., Ke, F.-C., Huang, C.-J., Kandaswami, C., Lee, P.-P. H. and Lee, M.-T. 2004. Transinactivation of the epidermal growth factor receptor tyrosine kinase and focal adhesion kinase phosphorylation by dietary flavonoids: effect on invasive potential of human carcinoma cells. Biochem. Pharmacol. 67: 2103–2114.

Liu, W.Y., Liou, S.S. Hong, T.Y. and Liu, I.M. 2017a. The benefits of the Citrus flavonoid diosmin on human retinal pigment epithelial cells under high-glucose conditions. Molecules, 22: doi: 10.3390/molecules22122251.

Liu, W.Y., Liou, S.S., Hong, T.Y. and Liu, I.M. 2017b. Protective effects of hesperidin (Citrus Flavonone) on high glucose induced oxidative stress and apoptosis in a cellular model for diabetic retinopathy. Nutrients, 9: doi: 10.3390/nu9121312.

Lota, M.L., de Rocca Serra, D., Tomi, F., Bessiere, J.M. and Casanova, J. 1999. Chemical composition of peel and leaf essential oils of *Citrus medica* L. and *C. limonimedica* Lush. Flavour Frag. J. 14: 161–166.

Mabberley, D. 2004. Citrus (Rutaceae): a review of recent advances in etymology, systematics and medical applications. Blumea-Biodiv. Evol. Biogeogr. Plants 49: 481–498.

Mabberley, D.J. 2008. Mabberley's plant-book: a portable dictionary of plants, their classifications, and uses, Cambridge University Press.

Manners, G.D. 2007. Citrus limonoids: analysis, bioactivity, and biomedical prospects. J. Agr. Food Chem. 55: 8285–8294.

Manners, G.D. and Breksa, A.P. 2004. Identifying citrus limonoid aglycones by HPLC-EI/MS and HPLC-APCI/MS techniques. Phytochem. Anal. 15: 372–381.

Manners, G.D. and Hasegawa, S. 1999. A new normal phase liquid chromatographic method for the analysis of limonoids in citrus. Phytochem. Anal. 10: 76–81.

Manthey, J.A. and Grohmann, K. 2001. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. J. Agr. Food Chem. 49: 3268–3273.

Matsubara, Y., Sawabe, A. and Iizuka, Y. 1990. Structures of new limonoid glycosides in lemon (*Citrus limon* Burm. f.) peelings. Agr. Biol. Chem. 54: 1143–1148.

Matsuda, H., Yoshikawa, M. and Kubo, M. 1998. Antinociceptive and anti-inflammatory activities of limonin isolated from the fruits of *Evodia rutaecarpa* var. *bodinieri*. Planta Med. 64: 339–342.

Matvienko, O.A., Lewis, D. S., Swanson, M., Arndt, B., Rainwater, D. L., Stewart, J. and Alekel, D. L. 2002. A single daily dose of soybean phytosterols in ground beef decreases serum total cholesterol and LDL cholesterol in young, mildly hypercholesterolemic men. Am. J. Clin. Nutr. 76: 57–64.

Miller, E.G., Porter, J.L., Binnie, W.H., Guo, I.Y. and Hasegawa, S. 2004. Further studies on the anticancer activity of *Citrus* limonoids. J. Agr. Food Chem. 52: 4908–4912.

Miyake, M., Ozaki, Y., Ayano, S., Bennett, R.D., Herman, Z. and Hasegawa, S. 1992. Limonoid glucosides in calamondin seeds. Phytochemistry 31: 1044–1046.

Monajemi, R., Oryan, S., Haeri-Roohani, A., Ghannadi, A. and Jafarian, A. 2010. Cytotoxic effects of essential oils of some Iranian Citrus peels. Iran. J. Pharm. Res. 183–187.

Moodley, V.E., Mulholland, D.A. and Raynor, M.W. 1995. Micellar electrokinetic capillary chromatography of limonoid glucosides from *Citrus* seeds. J. Chromatogr. A. 718: 187–193.

Murakami, A., Shigemori, T. and Ohigashi, H. 2005. Zingiberaceous and citrus constituents, 1′-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264. 7 murine macrophages through different modes of action. J. Nutr. 135: 2987S–2992S.

Njoroge, S., Ukeda, H., Kusunose, H. and Sawamura, M. 1994. Volatile components of Japanese yuzu and lemon oils. Flavour Frag. J. 9: 159–166.

Njoroge, S.M., Koaze, H., Mwaniki, M., Minh Tu, N. and Sawamura, M. 2005. Essential oils of Kenyan Citrus fruits: volatile components of two varieties of mandarins (*Citrus reticulata*) and a tangelo (*C. paradisi* × *C. tangerina*). Flavour Frag. J. 20: 74-79.

Nogata, Y., Sakamoto, K., Shiratsuchi, H., Ishii, T., Yano, M. and Ohta, H. 2006. Flavonoid composition of fruit tissues of citrus species. Biosci. Biotechnol Biochem. 70: 178–192.

Nordby, H.E. and Nagy, S. 1981. Chemotaxonomic study of neutral coumarins in roots of Citrus and Poncirus by thin-layer, gas-liquid and high-performance liquid chromatographic analyses. J. Chromatogr. A. 207: 21–28.

Normen, L., Johnsson, M., Andersson, H., Van Gameren, Y. and Dutta, P. 1999. Plant sterols in vegetables and fruits commonly consumed in Sweden. Eur. J. Nutr. 38: 84-89.

Ortiz, J.M. 2002. Botany: taxonomy, morphology and physiology of fruits, leaves and flowers. Citrus. 16–35.

Ozaki, Y., Fong, C.H., Herman, Z., Maeda, H., Miyake, M., Ifuku, Y. and Hasegawa, S. 1991. Limonoid glucosides in citrus seeds. Agr. Biol Chem. 55: 137–141.

Paulini, H., Popp, R., Schimmer, O., Ratka, O. and Röder, E. 1991. Isogravacridonchlorine: a potent and direct acting frameshift mutagen from the roots of *Ruta graveolens*. Planta Med. 57: 59-61.

Potter, S.M. 1995. Overview of proposed mechanisms for the hypocholesterolemic. J. Nutr. 125: 606S.

Raman, G., Cho, M., Brodbelt, J.S. and Patil, B.S. 2005. Isolation and purification of closely related Citrus limonoid glucosides by flash chromatography. Phytochem. Anal. 16: 155–160.

Reuther, W., Webber, H.J. and Batchelor, L.D. 1967. History, World Distribution, Botany, and Varieties, University of California, Division of Agricultural Sciences.

Robards, K., Li, X., Antolovich, M. and Boyd, S. 1997. Characterisation of citrus by chromatographic analysis of flavonoids. J. Sci. Food Agr. 75: 87–101.

Ruberto, G., Renda, A., Tringali, C., Napoli, E.M. and Simmonds, M.S. 2002. Citrus limonoids and their semisynthetic derivatives as antifeedant agents against *Spodoptera frugiperda* Larvae. A structure−activity relationship study. J. Agr. Food Chem. 50: 6766–6774.

Schnitzler, P., Schuhmacher, A., Astani, A. and Reichling, J. 2008. *Melissa officinalis* oil affects infectivity of enveloped herpesviruses. Phytomedicine. 15: 734–740.

Smeriglio, A., Alloisio, S., Raimondo, F.M., Denaro, M., Xiao, J., Cornara, L. and Trombetta, D. 2017. Essential oil of *Citrus lumia* Risso: Phytochemical profile, antioxidant properties and activity on the central nervous system. Food. Chem. Toxicol. 17: 30801–30803.

Singh, G., Kapoor, I., Singh, O., Leclercq, P. and Klinkby, N. 1999. Chemical constituents of peel and leaf essential oils of *Citrus medica* L. J. Essent. Oil Bear. Pl. 2: 119–125.

Sun, C., Li, X., Xu, C., Zhang, S., Chen, K., Chen, Q. and Liu, C. 2007. Determination of 9 (10 H)-Acridone by HPLC with Fluorescence Detection. J. Liq. Chromatogr. Rel. Technol. 30: 245–254.

Takeuti, N., Kasma, T., Aida, Y., Oki, J., Maruyama, I., Watanabe, K. and T obinaga, S. 1991. Pharmacological activities of the prenylcoumarins, developed from folk usage as a medicine of *Peucedanum japonicum* THUNB. Chem. Pharm. Bull. 39: 1415–1421.

Tanaka, T., Kohno, H., Tsukio, Y., Honjo, S., Tanino, M., Miyake, M. and Wada, K. 2000. Citrus limonoids obacunone and limonin inhibit azoxymethane-induced colon carcinogenesis in rats. Biofactors. 13: 213–218.

Tanaka, T., Maeda, M., Kohno, H., Murakami, M., Kagami, S., Miyake, M. and Wada, K. 2001. Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. Carcinogenesis. 22: 193–198.

Teng, C.-M., Li, H.-L., Wu, T.-S., Huang, S.-C. and Huang, T.-F. 1992. Antiplatelet actions of some coumarin compounds isolated from plant sources. Thromb. Res. 66: 549–557.

Tian, Q., Li, D., Barbacci, D., Schwartz, S. J. and Patil, B. S. 2003. Electron ionization mass spectrometry of citrus limonoids. Rapid Commun. Mass Sp. 17: 2517–2522.

Tian, Q., Miller, E. G., Ahmad, H., Tang, L. and Patil, B. S. 2001. Differential inhibition of human cancer cell proliferation by citrus limonoids. Nutr. Cancer. 40: 180–184.

Tian, Q. and Schwartz, S. J. 2003. Mass spectrometry and tandem mass spectrometry of citrus limonoids. Anal. Chem. 75: 5451–5460.

Tripathi, P., Dubey, N. and Shukla, A. 2008. Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*. World J. Microbiol Biotechnol. 24: 39–46.

Tusa, N., Abbate, L., Renda, A. and Ruberto, G. 2007. Polyphenols distribution in juices from Citrus allotetraploid somatic hybrids and their sexual hybrids. J. Agr. Food Chem. 55: 9089–9094.

Vikram, A., Jayaprakasha, G. and Patil, B.S. 2007. Simultaneous determination of citrus limonoid aglycones and glucosides by high performance liquid chromatography. Anal. Chim. Acta 590: 180–186.
Wink, M. 1999. Functions of plant secondary metabolites and their exploitation in biotechnology, Taylor & Francis.
Wink, M. 2008. Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. Curr. Drug Metab. 9: 996–1009.
Wolters, B. and Eilert, U. 1981. Antimicrobial substances in callus cultures of *Ruta graveolens*. Planta Med. 43: 166–174.
Wu, T.-S. and Furukawa, H. 1982. Biological and phytochemical investigation of *Clausena excavata*. J. Nat. Prod. 45: 718–720.
Yang, M.-S., Wu, T.-S. and Wang, C.-H. 1987. Citracridone-I: A new antispasmodic from root barks of *Citrus depressa*. Planta Med. 53: 143–147.
Yu, J., Wang, L., Walzem, R.L., Miller, E.G., Pike, L.M. and Patil, B.S. 2005. Antioxidant activity of citrus limonoids, flavonoids, and coumarins. J. Agr. Food Chem. 53: 2009–2014.
Ziegler, H. and Spiteller, G. 1992. Coumarins and psoralens from Sicilian lemon oil (*Citrus limon* (L.) Burm. f.). Flavour Frag. J. 7: 129–139.
Zobel, A.M., Wang, J., March, R.E. and Brown, S.A. 1991. Identification of eight coumarins occurring with psoralen, xanthotoxin, and bergapten on leaf surfaces. J. Chem. Ecol. 17: 1859–1870.

16

Pharmacological Properties of Extracts and Compounds Isolated from *Platonia insignis* Mart.—A Perspective for Developing Phytomedicines

Antonio do Nascimento Cavalcante,[1,2] *Chistiane Mendes Feitosa,*[1,3,*] *Layana Karine Farias Lima,*[3] *Ronaldo dos Santos Sousa Júnior*[1] and *Ana Gabriela Sousa Alencar*[1]

Introduction

The increasing use of plants and herbal extracts with therapeutic purposes curing or preventing several diseases has made these plants' extractivism an indispensable choice in national agriculture and popular or traditional medicine. The medicines represent the support of small and medium national pharmaceuticals industries.

Among the cultivated medicinal plants, there is a very good amount of exotic species; however, many of the native plant species are used by the population based on chemical and pharmacological researches, or on empirical or traditional knowledge.

Since the civilization's earliest days, plant species have been a main source of resources enabling accumulation and learning based on the information experience about the environment through the constant observation of natural phenomena in order to improve the conditions of feeding and, mainly, the search for a cure of many diseases, thus demonstrating a close relation between medicinal plants' use and their own evolution. There are reports existing of the use of vegetal extracts as medicines between 4500 and 1600 B.C. (Miguel and Miguel 2000, Rokaya et al. 2010, Allabi et al. 2011). Miraculous powers were attributed to sorcerers, shamans, magicians, healers, among others who had the known—wisdom of the power of drugs and/or poisons. Through this empirical (non-scientific) method it became possible to accumulate knowledge about the use of medicinal plants with the purpose of prevention, treatment, and

[1] Department of Chemistry, Federal University of Piauí, Ininga, 64049-550, Teresina, PI, Brazil.
[2] Institute of Education, Science and Technology of Maranhão, 65760-000, Presidente Dutra, MA, Brazil.
[3] Department of Pharmacy, Federal University of Piauí, 64049-550 Teresina, PI, Brazil.
* Corresponding author: chistiane@ufpi.edu.br

cure of several diseases; being a referential and inexhaustible source of new compounds in the search for phytomedicines (Corrêa et al. 2003, Leonti et al. 2010).

Until the 19th century, therapeutic resources were constituted predominantly by plants and their extracts. These last ones represented homemade medicine used at that time. The use of these extracts for therapeutic purposes were anchored in popular and scientific knowledge. In this context, medicinal plants were used in many ways and in different forms such as: isolated active substances suppliers, such as total extracts, purified or selected extracts, as a drug in the preparation of infusions or decoctions (Simões et al. 2000).

In this matter, Brazil stands out as a country with great research potential on plant species, since it has the largest and richest biodiversity on the planet (Nascimento 2013). One medicinal plant family that has aroused a great interest of the scientific community due to the promising chemical and biological properties is the family Clusiaceae (Noldin et al. 2006, Nualkaew et al. 2012). This family comprises approximately 1000 subordinated species to 47 genera, distributed in tropical and subtropical climate regions of the planet and a genus that reaches temperate regions. Of these genera, nine have about 90 species that are edible (Barroso et al. 2002, Costa Júnior 2011a). Furthermore, many of these species are widely used in popular medicine for pain treatment, infection, inflammation, and ulcer control (Noldin et al. 2006).

Regarding chemosystematics, plants belonging to this family are mainly composed by the following secondary metabolites: coumarins, xanthones, steroids, triterpenes and flavonoids; these natural metabolites have led to great interest from several researchers, especially from the medical field, since many of these substances are endowed with important biological activities. Many of these natural metabolites have shown some activities such as antidepressant, antioxidant, antifungal, cytotoxic, anti-HIV and antibacterial (Monache et al. 1988, Gustafson et al. 1992, Noldin et al. 2006, Costa Júnior 2011a).

Studies about the chemical composition of the family Clusiaceae's plants have shown that they are also rich in polyisoprenylated benzophenones (Costa Júnior 2011a).

This chapter describes *Platonia insignis* Mart. including its economic importance. In addition, it makes a general overview on the biological-pharmacological properties as well as the main constituents found in this plant.

Platonia insignis Mart. (bacurizeiro)

The bacurizeiro is a plant species that belongs to the family Clusiaceae, subfamily *Clusioideae* and *Platonia* genus. It is a monotypic species, classified as *Platonia insignis* Mart. (Braga 1976). It is composed of 1000 species and 47 genera distributed in tropical and subtropical regions of the world. The term *Platonic* is an *Platan*'s attribute, a Greek philosopher, and insignis, means notable, significant, important, great, in other words, something that draws much attention, in reference to the physical structure and utility of the plant, as well as its size, flavor, and fruit aroma (Barroso et al. 2002, Moura et al. 2007).

The bacurizeiro is a large fruit tree, with a height from 15 to 25 meters and a diameter up to 100 cm (Cavalcante 1988). This tree is native to the Amazon region of Brazil and Guyana, but is also found in Colombia and Paraguay, always occurring in open undergrowth areas, clearings and, mainly, secondary vegetation, rarely found in dense primary forest (Cavalcante 1988, Mourão and Beltrati 1995, Chitarra and Chitarra 2005, Fontenele et al. 2010). In Brazil, the dissemination center is the state of Pará, where a wide range of fruit shape, size, pulp yield and quality is found, besides other characteristics of economic interest; it can also be find in the states of Maranhão, Mato Grosso, Piauí and Goiás (Ferreira et al. 1987, Silva and Donato 1993, Villachica et al. 1996, Aguiar et al. 2008). In the state of Piauí, the bacurizeiro is found in limited lands to the north in the cities of Murici dos Portelas, Amarante and Palmeirais. It is commonly known as "bacuri", "bacuri-açu", "pakoori", and "wild mamme aple" in Guyana, "pakoelie" and "geelhart" in Suriname, "parcori" in French Guiana and "matazona" in Ecuador (Mourão and Beltrati 1995).

The bacuri is a large fruit bacurizeiro, round, with a thick rind and it has a yellowish color as it can be observed in Fig. 16.1, inside the bark there is pulp, which has a whitish color, being viscous with very pleasant odors and flavors (Fontenele et al. 2010). The fruit shows great potential, not only from the point of view of its industrial processing, in ice cream preparation, creams, soft drinks, jams and jellies; but also for *in nature* consumption (Silva et al. 2010). When it is in the process of ripening, it exudes a soft and fragrant odor and is rich in terpenes (Calvazara 1970, Alves and Jennings 1979).

Fig. 16.1 Fruits and seeds of *P. insignis* Mart. Source: Adapted by Costa Júnior 2011a.

In general, the number of seeds per fruit is from one to five, with predominance from two to four seeds (Villachica et al. 1996). In abnormal cases, fruits containing up six seeds can be find (Mourão 1992) or, in other cases without seeds (Calzavara 1970). According to Teixeira et al. (2005), bacuri is a non-climacteric fruit, which means that it only ripens when bound to the plant and in most trees, the fruits fall when ripe, so that harvesting fruits consists of collecting them from the soil (Fao 1987). The expression "bacuri" is from Tupi origin which means: "to fall" and curi: "soon", in other words, it only falls when it matures, indicating that it has ripened completely. After this phase, they do not improve their sensorial and nutritional characteristics, although a slight softening and loss of green color can be noticed (Nagy et al. 1990, Souza et al. 2001).

In Brazil the pulp has a very pleasant odor and taste, which is popularly accepted, both in the "*in nature*" way and in the preparation of sweetmeats, pies, jams, juices, jellies, and ice creams. The shell can also be used to make sweetmeats, creams, and ice creams, increasing fruit yield, and consequently adding values. This process must occur after the separation of the existing resin from this part of the fruit (Aguiar 2006). The growing demand for the fruit has stimulated producers to establish growing areas. The seeds of bacuri fruit have no utility in food, however, they can be used in the production of soap or the production of bacuri grease. The oil extracting process occurs with great difficulty once the seeds are soaked in water for more than a year and then boiled, the oil being removed from the surface of the boiling water. The bacuri butter makes the skin look golden, within a few minutes after its application, it is absorbed and the skin is left with a velvety touch, besides removing spots, and diminishing scars (Ferreira 2008, Moraes and Gutjahr 2009, Costa Júnior 2011a).

Pharmacological activities of *Platonia insignis* Mart.

Species from the family Clusiaceae such as *P. insignis* Mart. (bacurizeiro) are important medicinal plants used in Brazilian popular medicine, mainly for eczema, herpes, gastrointestinal diseases, dermatitis, schistosomiasis, leishmaniasis and malaria treatments. Studies involving the biological-pharmacological properties of this species can be seen in literature and most of these studies are especially focused on the seeds vegetal extracts of *P. insignis* Mart. Vegetal extracts or fats have shown important pharmacological activities such as: anti-inflammatory, antimalarial, antihypertensive, antidiabetic, immunomodulatory-respiratory, antiviral, antitumor, antidepressant, anti-allergic, anti-mutagenic and anti-oxidant effects (Bilanda et al. 2010, Costa Júnior et al. 2013a). In addition, these extracts are widely used in popular medicine for the treatment of various diseases such as: diarrhea, skin problems, earaches, spider and snakes' bites, rheumatism, arthritis and even as healing (Moraes and Gutjahr 2009, Costa Júnior et al. 2013a, Costa Júnior et al. 2013b).

The "bacuri grease" obtained from the seeds oil is commonly used in popular medicine as healing for burns and in the treatment of dermatological diseases (Agra et al. 2007). *In vivo* assays demonstrated its potential to accelerate the healing of rats' skin wounds (Santos et al. 2013, Feitosa et al. 2016). Popularly, the seeds' decoction is used for diarrhea treatment, while the seed`s oil is used both against spider and snakes' bites and in the treatment of skin problems, otitis, rheumatism, and arthritis (Agra et al. 2008, Mendes et al. 2014).

Pharmacological studies demonstrated that the ethyl acetate fraction from the *P. insignis* Mart. seeds have anticonvulsant activity potential in rats induced by pilocarpine (Costa Júnior et al. 2011b).

Through the leishmanicide property of the *P. insignis* Mart. seeds' extracts a patent order was requested (Citó et al. 2011), based on hexane activities, dichloromethane and ethyl acetate, and especially in isolated tautomerics substances, garcinielliptona FC (GFC) (Fig. 16.2), which presented a potential leishmanicidal activity against the *Leishmania amazonensis* promastigotes forms (Costa Júnior et al. 2013a, 2013b).

Studies analyzing the toxicological profile of GFC in animals, after the administration of Swiss mice treated by oral and intraperitoneal ways, showed that it did not produce toxic effects as evidenced by the absence of toxicity or mortality signs in the animals during the experimental period. However, a study by Prado et al. (2017) investigating the cytotoxic, genotoxic, and mutagenic effects of GFC, showed that GFC concentrations above 50 μg mL^{-1} were cytotoxic during the experimental time. No GFC concentration was mutagenic or genotoxic in the salmonella/microsome and comet assays.

The *in vitro* anti-oxidant property of bacuri has been commonly reported through the loss of free radicals. This exhibits a possible protective action against the appearance and/or development of degenerative processes associated with several types of diseases (Rufino et al. 2010, Vieira et al. 2011, Costa Júnior et al. 2013a).

The research conducted by Santos Júnior et al. (2010) allows us to suggest a potential healing activity for the *P. insignis* Mart. seeds oil in their experiments using "bacuri grease" to heal cutaneous wounds in the dorsal region of rats. The results showed that the lard was efficient in the treatment and the treated animals showed considerable re-epithelialization.

Mendes (2013) tested the healing activity of a pasty pharmaceutical formulation containing the compound 1,3-distearyl-2-oleyl glycerol (TG1) (Fig. 16.3), a triglyceride isolated from the hexanic extract of *P. insignis* Mart. seeds in Wistar rats. Macroscopic and histological analysis of the mice's wounds treated with the cream containing TG1 demonstrates a possible healing activity of the compound.

In studies performed by Mendes et al. (2014), the ethyl acetate fraction of the ethanolic extract from the bark of bacuri promoted a powerful hypotensive effect, related to α-adrenergic receptors.

Evaluating the *in vitro* anti-oxidant potential of *P. insignis* Mart. hexanic extract and its β-cyclodextrin inclusion complex against lipid peroxidation (2-thiobarbituric acid–TBARS formation inhibition); removing hydroxyl radical, and nitrite radical production *in vivo* inhibition, both extract and inclusion complex could inhibit lipid peroxidation by reducing the amount of TBARS, of hydroxyl radical and nitrite production (Nascimento et al. 2015).

Results reported by Costa Júnior et al. (2013b) indicated that the ethyl acetate and dichloromethane fractions of *P. insignis* ethanolic extract showed antioxidant activities *in vitro*, measured by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) *in vivo*

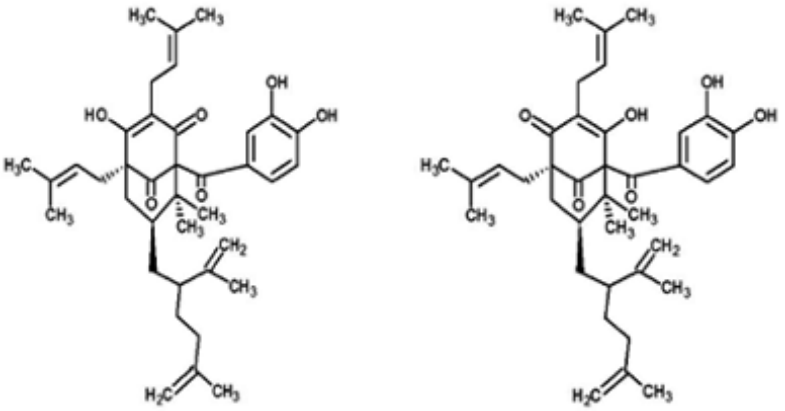


Fig. 16.2 Chemical structure of the garcinielliptona tautomerics (1/1a). Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

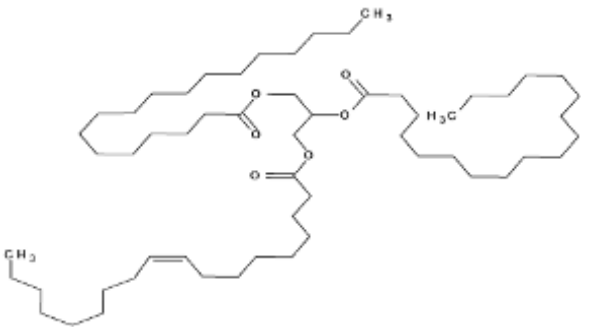


Fig. 16.3 Chemical structure of 1,3-distearyl-2-oleoyl glycerol molecular structures were drawn with ChemDraw13. (Perkin Elmer).

assays, measured by protective effects against H_2O_2-induced cytotoxicity in *Saccharomyces cerevisiae* stranins; leishmanicidal effect and genotoxicity in V79 cells where the compounds α-mangostin and γ-mangostine are responsible for these actions.

Other biological activities of *P. insignis* Mart. seeds reported in the literature are antimicrobial, against *Saccharomyces cerevisiae* strains; cytotoxicity in *Artemia salina*; genotoxic effect on Chinese hamster lung fibroblasts (V79); pilocarpine-induced anticonvulsive effect; healing; anti-inflammatory activity and stimulatory effect on mice's Central Nervous System (CNS) (Costa Júnior et al. 2010, Santos Júnior et al. 2010, Costa Júnior et al. 2011a, 2011b, 2011c, Costa Júnior et al. 2013a, 2013b). Table 16.1 lists the biological activities described in the literature for all parts of the bacuri fruit.

Table 16.1 Biological activities of the bacuri fruit parts.

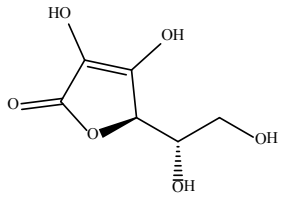
Part	Biological activities	References
Seeds	Diarrhea, skin problem, Earaches; Spider, snakes and insect bites; Rheumatism and arthritis; Healing; leishmanicidal activity; Antioxidant; Anti-inflammatory; cytotoxicity in *Artemia salina*; genotoxic effect on Chinese hamster lung fibroblasts; Anticonvulsive effect induced by pilocarpine; Protective effect, reducing lipid peroxidation; Anticonvulsive activity; CNS stimulatory effect in mice	Moraes and Gutjahr 2009, Costa Junior et al. 2010, Santos Júnior et al. 2010, Costa Júnior et al. 2013a, 2013b, Costa Júnior et al. 2011a, 2011b
Pulp	Antioxidant activity	Rufino et al. 2010
Trunk bark	Eczema treatment, herpes virus and dermatitis treatment	Shanley and Medina 2005
Fruit peel	Hypotensive activity	Mendes et al. 2014

Source: Adapted from Klenicy et al. 2014

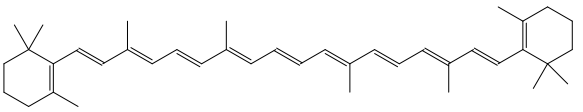
Chemical composition of *Platonia insignis* Mart.

The *P. insignis* Mart. is a vegetal species composed mostly of terpenes, xanthones and phenols. Thus, the interest of this species has aroused the food industry due the potential of its biological activities found (Yamaguchi et al. 2014). The *Platonia* genus is quite abundant in several natural substances (metabolites) such as xanthones (euxanthones), fatty acids, and triacylglycerols (Hilditch and Pathak 1949, Roberts 1961, Bentes et al. 1986).

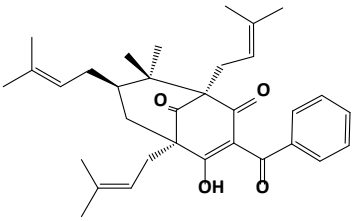
Research on *P. insignis* fruit pulp detected the ascorbic acid (1) of Fig. 16.4 and the presence of polyphenols as the main bioactive compounds (Clerici and Carvalho-Silva 2011). The xanthones class is responsible for several pharmacological properties of great importance, such as: antitumor, anti-



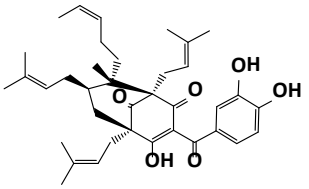
(1)



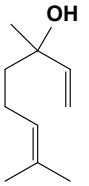
(2)



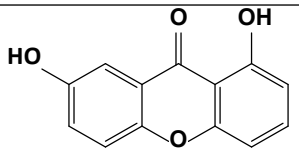
(3)



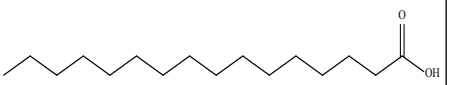
(4)



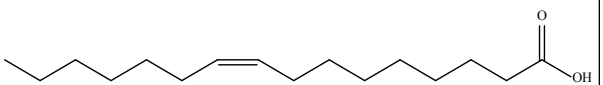
(5)



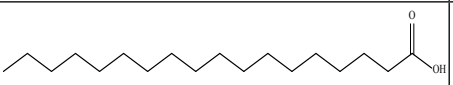
(6)



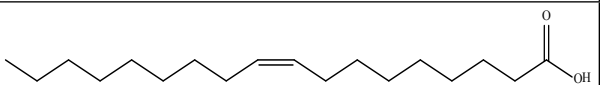
(7)



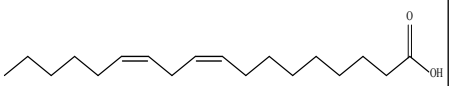
(8)



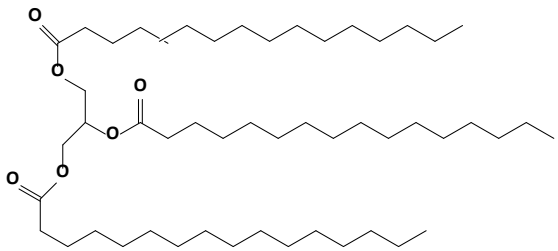
(9)



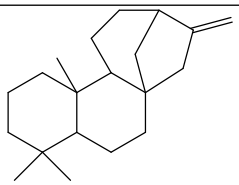
(10)



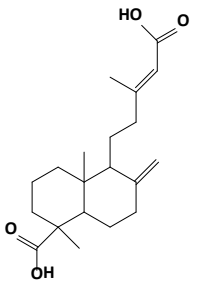
(11)



(12)



(13)



(14)

Fig. 16.4 contd....

...Fig. 16.4 contd.

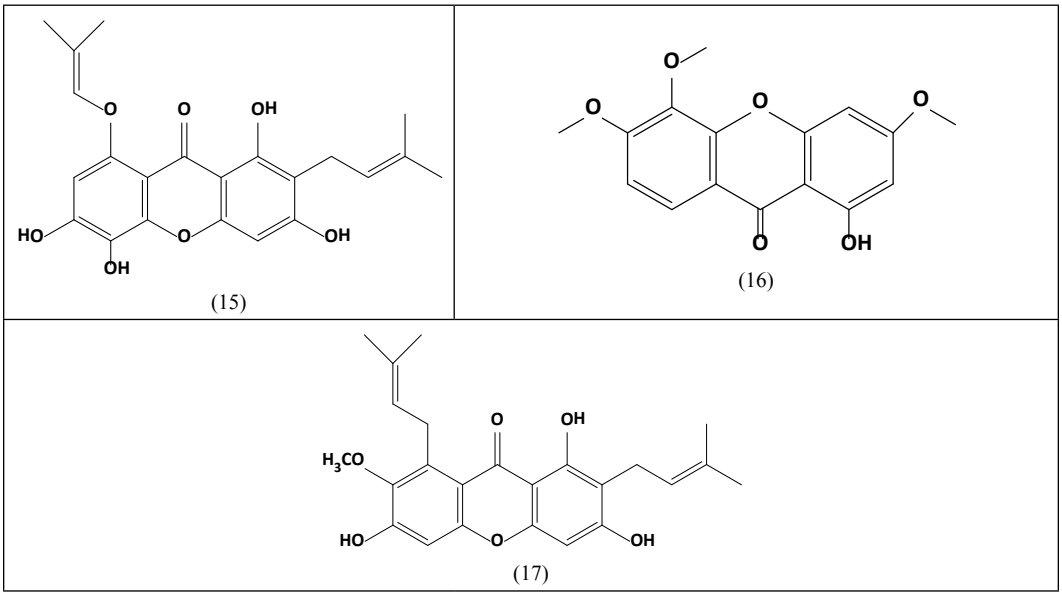


Fig. 16.4 Shows some of the *P. insignis* Mart. (Bacurizeiro) constituents chemical structures.

inflammatory, antithrombotic, antimicrobial and neuropharmacological effects, thus inducing the formation of neurites in nervous tissue (Mak et al. 2000, Ha et al. 2006).

The search for components with active biological activities of the family Clusiaceae, Costa Júnior et al. (2011b) investigated bioactive components of *P.insignis* seeds and isolated tautomerics compounds from a polyisoprenylated benzophenone, garcinielliptona (1/1a) (Fig. 16.2). These substances have been identified by spectroscopic methods. Another isolated compound from the same *P. insignis* seed extract was the triacylglyceride, 1,3-distearyl-2-oleyl glycerol (Fig. 16.3). The fruit of this species is also rich in β-carotene (2) (Rodriguez-Amaya et al. 2008).

Phytochemical studies of pericarp hexanic extracts and bacupari seeds, *Garcinia brasiliensis* (species from the same bacuri family), methanolic extract showed, respectively, the presence of prenylated benzophenones, 7-epiclusianonae (3) and guttiferone-A (4). Both vegetal extracts and isolated compounds were pointed out as potential herbal remedies for diseases caused by *gram* negative microorganisms (Naldoni et al. 2009). The acylphloroglucinol substance, which is a polyclenyl polycyclic isolated from the Clusiaceae family, has aroused the interest of many researchers, because this substance presents an important range of pharmacological properties: antimicrobial (Oliveira et al. 1999), leishmanicide (Pereira et al. 2010), antidepressant (Cuesta-Rubio et al. 2002), antioxidant (Cuesta-Rubio et al. 2002, Ciochina and Grossman 2006, Chen et al. 2010), cytotoxic (Baggett et al. 2005), antiretroviral (Piccinelle et al. 2005), anti-inflammatory (Weng et al. 2004) and antitumoral (Zeisser-Labauébe et al. 2006, Henry et al. 2009).

Studies on the bacuri pulp identified the presence of chemical constituents with antioxidant proprieties such as vitamins C (ascorbic acid), vitamin E (tocopherols), flavonoids, anthocyanins, and polyphenols, also, the presence of glutamine and glutamic acid (as major aminoacids). Saccharides (glucose, fructose and sucrose) and metals (Na, K, Ca, Mg, Fe, Zn and Cu) were observed in higher amounts than those found in Amazon region fruits, such as araçá-boi and cupuaçu (Rogez et al. 2004, Rufino et al. 2010). The analysis of volatile compounds studies of *P. insignis* Mart. showed the presence of terpene alcohols, linalool being the the most abundant (5) (Alves and Jennings 1979, Boulanger et al. 1999, Rogez et al. 2004, Franco and Janzantti 2005). *P. insignis* shells showed a high euxanthone content (6) (1.3%), a yellow crystalline substance with melting point 240°C that undergoes sublimation easily (Roberts 1961).

In phytochemical studies of seeds lipid fractions of *P. insignis* Mart. confirmed the presence of fatty acids such as palmitic acid (7) (44.2%), palmitoleic (8) (13.2%), stearic (9) (2.3%), oleic (10) (37,8%) and

linoleic (11) (2.5%), besides 10% tripalmitin (12), indicating it is a good alternative for the oil industry (Bentes et al. 1986).

In the *P. insignis* Mart. low polarity fraction detected the presence of cauran and labdane diterpenes skeletons, caura-16-ene (13) and E-labda-8 (20), 13-diene-15,19-dioic acid (14), respectively. These diterpene skeletons are normally associated with pharmacological activities, since there are antibacterial and anti-inflammatory activities reports for some diterpenes, such as those found in copaiba (*Copaifera* sp.) and *Eperua* species fixed oils and resins (Leandro et al. 2012). In the medium polar fraction, analyzed by Gas Chromatography coupled to Mass Spectrometer (GC-MS), detected the presence of the xanthones: 1,3,5,6-tetrahydroxy-2,2-(2-methylbut-3-en-2-yl)-7-(3-methylbut-2-enyl)-xanthen-9-one (15) (Gamma-mangostin), associated with 1-hydroxy-3,5,6-trimethoxyxanthen-9-one (16) and 1,3,6-trihydroxy-7-methoxy-2,8-bis- (3-methylbut-2-enyl)-xanthen-9-one (17) (Costa Júnior et al. 2012).

The volatile substances present in *P. insignis* are basically concentrated on the shell. Through the shell supercritical fluid extraction, the following compounds were detected: fatty acids: palmitic, oleic, linoleic, linolenic, stearic, caprylic and myristic; the alcohols found were: linalool, 3,7-dimethyl-oct-1-ene-3,7-diol and terpineol; in addition to linalool oxide; eugenol ether; the following hydrocarbons: bisabolene, 2-methylheptane and nonacosane, as well as trimethyl citrate (Monteiro et al. 1997).

Table 16.2 shows the pharmacological properties of *P. insignis* Mart. main bioactive compounds.

Figure 16.4 shows some of the *P. insignis* Mart. (Bacurizeiro) constituents chemical structures.

Table 16.2 Pharmacological properties of *P. insignis* mart. Main bioactive compounds.

Bioactive compounds	**Pharmacological Properties**	**References**
Xanthones	Antitumoral Anti-inflammatory Antithrombotic Antimicrobial Neuropharmacological	Roberts 1961 Mak et al. 2000 Ha et al. 2006
Fatty acids	Accelerates healing	Calder 2003 Cardoso et al. 2004 Hatanaka and Curi 2007 Costa Júnior et al. 2011a
Acylphloroglucinol Polycyclic polyprenylated	Antimicrobial Leishmanicide Antidepressant Antioxidant Cytotoxic	Oliveira et al. 1999, Cuesta-Rubio et al. 2002, Baggett et al. 2005 Pereira et al. 2010 Ciochina and Grossman 2006 Chen et al. 2010
Euxantone	Antiretroviral Anti-inflammatory Antitumoral Pro-oxidant effect on DNA	Piccinelli et al. 2005 Weng et al. 2004 Zeisser-Labouebe et al. 2006 Henry et al. 2009 Wu et al. 2008
Garcinielliptona FC	Anti-inflammatory Vasodilator Neurrite promoter *In vitro* antioxidant action by TBARS methods, hydroxyl radicals (OH) sequestration and nitric oxide (NO) Cytotoxic activity Leishmanicide	Harborne et al. 1999 Fang et al. 2006 Ha et al. 2006 Naidu et al. 2007 Costa Júnior et al. 2013a, Costa Júnior et al. 2011a
TG1	Acetylcholinesterase enzyme inhibitory action Healing	Santos 2012, Feitosa et al. 2016, Mendes 2013

Source: Adapted from Santos et al. 2013

Anticholinesterase activity test

The inhibition of the acetylcholinesterase enzyme, AChE, a key enzyme in the acetylcholine degradation, serve as a strategy for various neurodegenerative diseases treatment such as Alzheimer's Disease (AD). There are some synthetic and natural rivastigmine-based medicine for the cognitive dysfunction and memory loss associated with AD treatment. These compounds have been known for an adverse effect, including gastrointestinal problems and disorders, associated with their bioavailability, which requires in finding more efficient acetylcholinesterase enzyme (AChE) inhibitors from natural resources (Mukherjee et al. 2007). AD is related to cognitive deficit and memory retention due to the cholinergic neurotransmission disjunction, another efficient treatment for this disease is based on the acetylcholine levels increase from the acetylcholinesterase (AChE) enzyme inhibition, that is the cholinergic hypothesis.

In the same context, the search for new AChE inhibitors present in natural products, such as *P. insignis* Mart. extracts, which may have fewer side effects to be used as phytotherapies in the future, has increased.

One of the frequently used assays to detect the anticholinesterase activity is the Ellman assay. Ellman et al. (1961) developed an assay through a photometric method which is able to measure the AChE activity in natural products, tissues extract, homogenates, cell suspensions, among others. The AChE use stands out as one of the fastest and most sensitive bioassays to select samples with anticholinesterase action.

The enzyme qualitative inhibition test is performed on silica gel chromatoplate. Caffeine and isolated substances or extracts in five concentrations are applied on the plate (positive standard) (image A of Fig. 16.5). The eluents used are: chloroform-methanol (9:1) (image B of Fig. 16.5). The plates are sprayed with a solution formed by mixing a 10 mL Tris (hydroxymethyl methane) buffer pH 8, 2,9 mg of acetylcholine iodide and 4 mg of 5,5'-dithiobis (2-nitrobenzoic acid) (image D of Fig. 16.5). After drying the plate, the AChE enzyme with concentration of 5 U mL^{-1} is sprayed. A positive result for the AChE (anticholinesterase activity) inhibition is obtained from the yellow chromatoplate with white halos (image E of Fig. 16.5), and it was necessary to proceed with this enzyme quantitative inhibition test.

Fig. 16.5 Ellman assays for TLC (A) sample application and positive pattern, (B) sample sowing, (C) sample eluted, (D) sample spray, (E) positive result of cholinesterase activity.

The AChE activity measurement can be performed according to the Ellman Method (1961) modified by Ingkaninan et al. (2001) for Thin Layer Chromatography (TLC). The principle of this method is the thiocholine production ratio measurement when the acetylthiocholine iodide substrate is hydrolyzed by the AChE. When thiocholine reacts with 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) produces the yellowish anion 5-thio-2-nitrobenzoate (Fig. 16.6). The yellow color production ratio is measured in a spectrophotometer (405 nm) or visualized in thin TLC by white spots on a yellow field. Alzheimer's disease is related to neurotransmitter acetylcholine decrease.

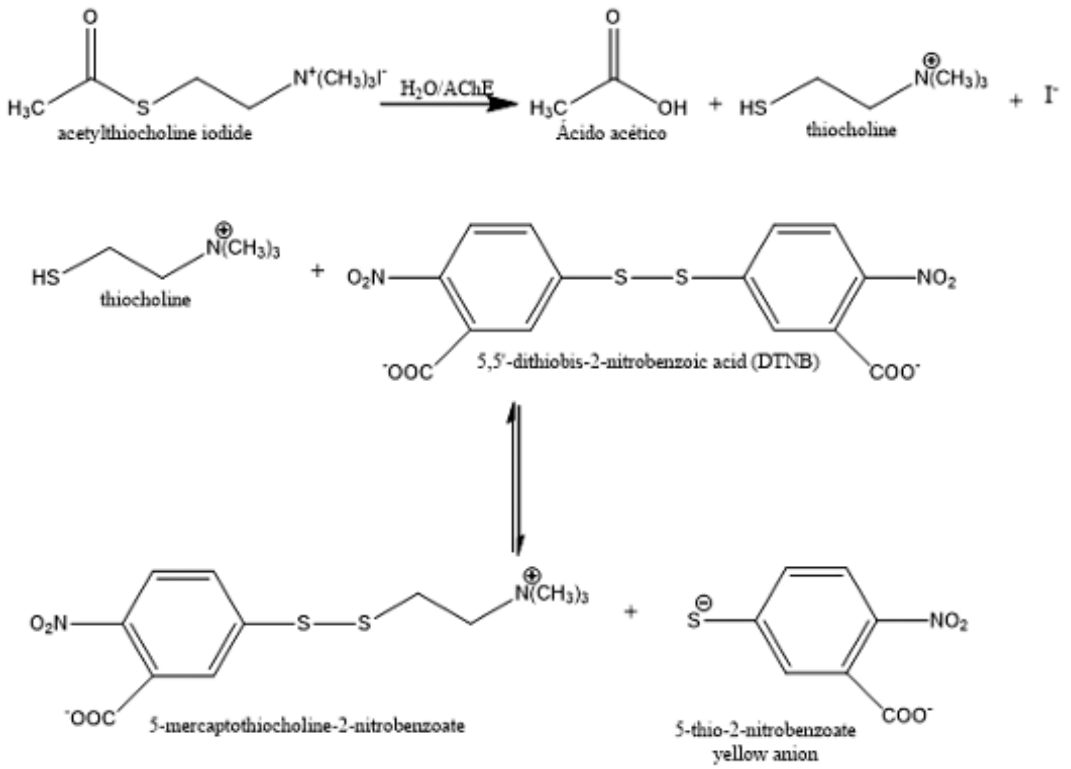


Fig. 16.6 Formation of the 5-thio-2-nitrobenzoate yellow anion resulting from the reaction between a thiocholine and the 5,5'-dithiobis-2-nitrobenzoate ion.

Conclusion

In the extract and isolated substances of *Platonia insignis* Mart. (bacurizeiro) phytochemical and pharmacological studies demonstrated that this species is a potential promising source for elaboration of possible drugs. Some of the pharmacological properties that were found in this plant's extract and isolated compounds were anti-inflammatory, antioxidant, anticonvulsive, healing and anticholinesterase. These characteristics are important for the preparation of drugs from this medicinal plant. In its chemical composition, bacuri presents classes of terpenes, xanthones and phenolic compounds as major constituents. Some of these classes of compounds present biological activities related to the ethnopharmacological use and activities found.

Thus, interest is aroused in the food industry for bacuri due to the potential biological-pharmacological activities found.

References

Agra, M.F., Freitas, P.F. and Barbosa-Filho, J.M. 2007. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Braz. J. Pharmacogn. 17: 114–40.

Agra, M.D.F., Silva, K.N., Basílio, I.J.L.D., Freitas, P.F.D. and Barbosa-Filho, J.M. 2008. Survey of medicinal plants used in the region Northeast of Brazil. Braz. J. Pharmacogn. 18(3): 472–508.

Aguiar, L.P., Figueiredo, R.W., Alves, R.E., Maia, G.A. and Souza, V.A.B.D. 2008. Physical and physico-chemical characterization of fruits from different genotypes of bacuri (*Platonia insignis* Mart.). Food Sci. Technol. 28: 2.

Aguiar, L.P. 2006. Quality and potential use of bacurís (*Platonia insignis* Mart.) From the Mid-North region. 122 p. Masters dissertation. Fortaleza-CE, Brazil.

Alves, S.M. and Jennings, W.G. 1979. Volatiles composition of certain Amazonian fruits. Food Chem. 4: 149–159.

Allabi, A.C., Busia, K., Ekanman, V. and Bakiono, F. 2011. The use of medicinal plants in self-care in the Agonlin region of Benin. J. Ethnopharmacol. 133: 234–243.

Barroso, G.M., Peixoto, A.L., Ichaso, C.L.F., Costa, C.G., Guimarães, E.F. and Lima, H.C. 2002. Sistemática de Angiospermas do Brasil Minas Gerais: Viçosa, p. 309.

Baggett, S., Protiva, P., Mazzola, E.P., Yang, H., Ressler, E.T., Basile, M.J., Weinstein, I.B. and Kennelly, E.J. 2005. Bioactive Benzophenones from *Garcinia xanthochymus* Fruits. J. Nat. Prod. 68: 354–360.

Bentes, M.H.S., Serruya, H., Rocha Filho, G.N., Godoy, R.L.O., Silva Cabral, J.A. and Soares Maia, J.G. 1986. Chemical study of bacuri seeds. Acta Amazon. 16: 363–368.

Bilanda, D.C., Dimo, T., Dzeufiet Djomeni, P.D., Bella, N.M.T., Aboubakar, O.B.F., Nguelefack, T.B., Tan, P.V. and Kamtchouing, P. 2010. Antihypertensive and antioxidant effects of *Allanblackia floribunda* Oliv. (Clusiaceae) aqueous. J. Ethnopharmacol. 128: 634–640.

Boulanger, R., Chassagne, D. and Crouzet, J. 1999. Free and bound components of Amazonian fruits.1: Bacuri. Flavour Frag. J. 14: 303–311.

Braga, R. 1976. Plantas medicinais do Nordeste, especialmente do Ceará: Mossoró: ESAM.

Calder, P.C. 2003. Long-chain n-3 fatty acids and inflammation: potential application in surgical and trauma patients. Braz. J. Med. Biol. Res. 36: 433–446.

Calzavara, B.B.G. 1970. Fruteiras: abieiro, abricozeiro, bacurizeiro, biribazeiro, cupuaçuzeiro. IPEAN, Séries Culturas da Amazônia, Belém. 63–68.

Cardoso, C.R.B., Souza, M.A., Ferro, E.A.V., Favoreto, J.R. and Pena, J.D.O. 2004. Influence of topical administration of n-3 and n-6 essential and n-9 nonessential fatty acids on the healing of cutaneous wounds. Wound Repair Regen. 12: 235–243.

Carvalho, J.E.U., Nascimento, W.M.O. and Muller, C.H. 1998. Características físicas e de germinação de sementes de espécies frutíferas nativas da Amazônia. Belém: Embrapa-CPATU, (Boletim de Pesquisa, 203), p. 18.

Cavalcante, P.B. 1988. Frutas comestíveis da Amazônia. 4. ed. Belém: Museu Paraense Emílio Goeldi. 48–50.

Chitarra A.B. and Chitarra, M.I.F. 2005. Pós-colheita de frutos e hortaliças: Glossário. Lavras, UFLA, p. 256.

Chen, X.Q., LI, Y., Cheng, X., Wang, K., He, J., Pan, Z.H., Li, M.M., Peng, L.Y., Xu, G. and Zhao, Q.S. 2010. Polycyclic polyprenylated acylphloroglucinols and chromone O-glucosides from Hypericum henryi subsp. Uraloides. Chem. Biodivers. 7: 196–204.

Clereci, M.T.P.S. and Silva, L.B.C. 2011. Nutritional bioactive compounds and technological aspects of minor fruits grown in Brazil. Food Res. Int. 44: 1658–1670.

Ciochina, R. and Grossman, R.B. 2006. Polycyclic polyprenylated acylphloroglucinols. Chem. Rev. 106: 3963–86.

Citó, A.M.G., Costa Junior, J.S., Santana, L.C.L.R., Freitas, R.M., Carvalho, F.A.A., Ferraz, A.B.F. and Saffi, J. 2011. Seeds oil activity of *Platonia insignis* Mart. as leishmanicide. Brasil. Patent: Industrial model. Registration number: PI1101608-6, deposit date: 04/15/2011.

Corrêa, A.D., Batista, R.S. and Quintas, L.E. 2003. Plantas Medicinais: do cultivo à terapêutica-contém formulação e modo de preparo de cosméticos. Petrópolis: Vozes. 6. Petrópolis-RJ, p. 103.

Costa Júnior, J.S., Feitosa, C.M., Citó, A.M.G.L., Freitas, R.M., Henriques, J.A.P. and Saffi, J. 2010. Evaluation of effects of ethanolic extract (EE) from *Platonia insignis* Mart. on pilocarpine-induced seizures. J. Biol. Sci. 10: 747–753.

Costa Junior, J.S., Ferraz, A.B.F., Filho, B.A.B., Feitosa, C.M., Citó, A.M.G.L., Freitas, R.M. et al. 2011a. Evaluation of *in vitro* antioxidant effects of garcinielliptona fc (GFC) isolated from platonia insignis Mart. J. Med. Plants Res. 5: 293–299.

Costa Júnior, J.S., Almeida, A.A.C., Tomé, A.R., Citó, A.M.G.L., Saffi, J. and Freitas, R.M. 2011b. Evaluation of possible anticonvulsant effects of ethyl acetate fraction from *Platonia insignis* Mart. on epilepsy models. Epilepsy Behav. 22: 678–684.

Costa Júnior, J.S., Ferraz, A., Feitosa, C.M., Citó, A.M.G.L., Saffi, J. and Freitas, R.M. 2011c. Evaluation of effects of dichloromethane fraction from *Platonia insignis* Mart. on pilocarpine-induced seizures. Braz. J. Pharmacogn. 21: 1104–1110.

Costa Júnior, J.S., Ferraz, A.B.F., Sousa, T.O., Silva, R.A.C., Lima, S.G., Feitosa, C.M., Citó, A.M.G.L., Freitas, R.M., Sperotto, A.R.M., Peres, V.F., Moura, D.J. and SAFFI, J. 2012. Investigation of biological activities of dichloromethane and ethyl acetate fractions of *Platonia insignis* Mart. seed. Basic Clin. Pharmacol.

Costa Junior, J.S., Almeida, A.A.C., Ferraz, A.B.F., Rossato, R.R., Silva, T.G., Silva, P.B.N., Militão, G.C.G., Citó, A.M.G.L., Santana, L.C.L.R., Carvalho, F.A.A. and Freitas, R.M. 2013a. Citotoxic and leishmanicidal properties of garcinielliptone FC, a prenylated benzophenone from *Platonia insignis*. Nat. Prod. Res. 27: 470–474.

Costa Júnior, J.S., Ferraz, A.B.F., Sousa, T.O., Silva, R.A.C., Lima, S.G., Feitosa, C.M., Citó, A.M.G.L., Cavalcante, A.A.C.M., Freitas, R.M., Speroto, A.R.M., Peres, V.F., Moura, D.J. and Saffi, J. 2013b. Investigation of biological activities of dichloromethane and ethyl acetate fractions of *Platonia insignis* Mart. seed. Basic Clin. Pharmacol. 112: 34–41.

Cuesta-Rubio. O., Frontana-Uribe, B.A., Ramirez-Apan, T. and Cardenas, J. 2002. Polyisoprenylated benzophenones in cuban propolis; biological activity of nemorosone. Z. Naturforsch. C 57: 372–378.

Ellman, G.L., Courtney, D.K. and Andres, V. Jr. 1961. Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88–95.

Fao. 1987. Especies forestales productoras de frutas y otros alimentos. Ejemplos de América Latina. Roma: SIDA. 167–170.

Fang, L.H., Um, Y.M., Lin, L.L., Xiao, P.G. and Du, G.H. 2006. Vasorelaxant effect of eu-xanthone in the rat thoracic aorta. Vasc. Pharmacol. 45: 96–101.

Feitosa, C.M., Cavalcante, A.N., Chaves, S.K.M. and Araújo, L.S. 2016. Medicinal plants of Brazil and Alzheimer's disease: Evolution in traditional use and pre-clinical studies. Asian J. Biomed. Pharm. Sci. 6: 01–13.

Ferreira, F.R., Ferreira, S.A.N. and Carvalho, J.D.U. 1987. Espécies frutíferas pouco exploradas, com potencial econômico e social para o Brasil.

Ferreira, M.S.G. 2008. Manejo da espécie *Platonia insignis* Mart – Bacurizeiro, em florestas secundárias da Amazônia Oriental: proposta para uma produção sustentável. doctoral thesis, Centro de Desenvolvimento Sustentável, University of Brasília, Brasília, DF. Brazil, p. 246.

Fontenele, M.A., Figueiredo, R.W., Maia, G.A., Alves, R.E., Sousa, P.H.M.D. and Souza, V.A.B.D. 2010. Conservação pós-colheita de bacuri (*Platonia insignis* Mart.) sob refrigeração e embalado em PVC. Rev. Ceres, Viçosa. 57: 292–296.

Franco, M.R.B. and Janzantti, N.S. 2005. Aroma of minor tropical fruits. Flavour Frag. J. 20: 358–371.

Gustafson, K.R., Blunt, J.W., Munro, M.H.G., Fuller, R.W., Mckee, T.C., Cardellina II, J.H., Mcmahon, J.B., Cragg, G.M. and Boyd, M.R. 1992. Theguttiferones, HIV-inhibitory benzophenones from *Symphonia globulifera*, *Garcinia livingstonei*, *Garcinia ovalifolia*, and *Clusia rosea*. Tetrahedron 48: 10093–10102.

Ha, W.Y., Wu, P.K., Kok, T.W., Leung, K.W., Mak, N.K., Yue, P.Y.K., Ngai, S.M., Tsai, S.N. and Wong, R.N.S. 2006. Involvement of protein kinase C and E2F-5 in euxanthone-induced neurite differentiation of neuroblastoma. Int. J. Biochem. Cell B. 38: 1393–1401.

Harbone, J.B., Baxter, H. and Moss, G.P. 1999. Phytochemical dictionary: a handbook of bioactive compounds from plants. 2. ed. London: Taylor & Francis, p. 893.

Hatanaka, E. and Curi, R. 2007. Ácidos graxos e cicatrização: uma revisão. Braz. J. Pharm. Sci. 88: 53–58.

Henry, G.E., Campbell, M.S., Zelinsky, A.A., Liu, Y., Bowen-Forbes, C.S., Li L., Nair, M.G., Rowley, D.C. and Seeram, N.P., 2009. Bioactive acylphloroglucinols from *Hypericum densiflorum*. Phytother. Res.: PTR. 23: 1759–1762.

Hilditch, T.P. and Pathak, S.P.S. 1949. The component glyceride of Bacury (*Platonia Insignis* Mart.) seed fat. J. Chem. Soc. 1949: 87–90.

Inghaninan, K., Bes, C.M., Van Der Heijden, R., Hofte, A.J.P., Karabatak, B., Irth, H., Rhee, I.K., Van De Meent, M., Ingnaninan, K. and Verpootte, R. 2001. Screening for acetylcholinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. J. Chromatogr. A 915: 217–223.

Klenicy, K.L., Yamaguchi, C.V.L.P., Lima, E.S. and Veiga Júnior, V.F. 2014. Química e farmacologia do bacuri (*Platonia insignis*). Sci. Amazon. 3: 39–46.

Leandro, L.M. and Veiga Júnior, V.F. 2012. O gênero Eperua Aublet: uma revisão. Sci. Amazon. 1: 14–22.

Leonti, M., Cabras, S., Weckerlec, C.S., Solinas, M.N. and Casu, L. 2011. The causal dependence of present plant knowledge on herbals—Contemporary medicinal plant use in Campania (Italy) compared to Matthioli (1568). J. Ethnopharmacol. 130: 379–391.

Mak, N.K., Li, W.K., Zhang, M., Wong, R.N.S., Tai, L.S., and Yung, K.K.L. 2000. Effects of euxanthone on neuronal differentiation. Life Sci. 66: 347–354.

Mendes, C.S.M. 2013. Delineamento de um fitomedicamento a partir de 1,3-diestearil-2-oleil-glicerol isolado de *Platonia insignis* Mart: avaliação da atividade cicatrizante. Masters dissertation-Pharmaceutical Sciences. Federal University of Piaui-. Teresina-Brazil, 80 p.

Mendes, M.B., Silva Filho, J.C., Sabino, C.K.B., Arcanjo, D.D.R., Sousa, C.M.M., Costa, I.C.G., Chaves, M.H., Oliveira, R.C.M. and Oliveira, A.P. 2014. Pharmacological evidence of α2-adrenergic receptors in the hypotensive effect of *Platonia insignis* Mart. J. Med. Food 17: 1079–1085.

Miguel, M.D. and Miguel, O.G. 2000. Desenvolvimento de Fitoterápicos. 1ª Ed. Robe editorial.

Monache, F.D., Monache, G.D., Moura Pinheiro, R. and Radics, L. 1988. Nemorosonol, a derivative of tricyclo-[4.3.1.03,7]-Decane-7-hydroxy-2,9-dione from Clusia nemorosa. Phytochemistry 27: 2305–2308.

Monteiro, A.R., Meireles, M.A., Marques, M.O.M. and Petenate, A.J. 1997. Extraction of the soluble material from the shells of the bacuri fruit (*Platonia insignis* Mart) with pressurized CO_2 and other solventes. J. Supercrit. Fluid. 11: 91–102.

Moraes, R.L.B. and Gutjahr, E. 2009. Química de Oleogenosas - Valorização da Biodiversidade Amazônica. German Technical Cooperation Agency.

Moura, M.C.C.L., Homma, A.K.O., Menezes, A.J.E.A., Carvalho, A.C.P.P., Ferreira, A., Benbadis, A.K., Muller, C.H., Ferreira, C.A.P., Cruz, C.D., Araujo, E.C.E., Matos, G.B., Almeida, H.J., Carvalho, J.E.U., Costa, J.T.A., Araújo, J.R.G., Macarenas, K.M., Vasconcelos, L.F.L., Aloufa, M.A.I., Martins, M.R., Innveco, R. and Souza, V.A.B. 2007. Bacuri: Agrobiodiversidade. São Luís: Interamerican Institute for Cooperation on Agriculture. 210 p.

Mourão, K.S.M. and Beltrati, C.M. 1995. Morfologia dos frutos, sementes e plântulas de *Platonia insignis* Mart. (Clusiaceae). I. Aspectos anatômicos dos frutos e sementes em desenvolvimento. Acta Amazon. 25: 11–32.

Mourão, K.S.M. 1992. Morfologia e desenvolvimento dos frutos, sementes e plântulas de *Platonia insignis Mart*. (Clusiaceae) *Platonia insignis* Mart. (Guttiferae). Master's dissertation – Institute of Biology, Universidade Estadual Paulista, Rio Claro-Brazil.

Nagy, S., Shaw, P.E. and Wardowski, W.F. 1990. Fruits of tropical and subtropical origin. Composition, properties and uses. Florida Science Source, Inc. ISBN 0944961002.

Naidu, M., Kuan, C.Y.K., Lo, W.L., Raza, M., Tolkovsky, A., Mak, N.K., Wong, R.N.S. and Keynes, R. 2007. Analysis of the action of euxanthone, a plant-derived compound that stimulates neurite outgrowth. Neuroscience 148: 915–924.

Naldoni, F.J., Claudino, A.L.R., Cruz Jr, J.W., Chavasco, J.K., Faria e Silva, P.M., Veloso, M.P. and Santos, M.H. 2009. Antimicrobial Activity of Benzophenones and Extracts from the Fruits of *Garcinia brasiliensis*. J. Med. Food 12: 403–407.

Nascimento, J.N. 2013. Complexo de inclusão do extrato hexânico de *Platonia insignis* Mart e β-ciclodextrina: caracterização e avaliação das atividades gastroprotetora e antioxidante *in vitro.* 137 p. Masters dissertation – Centro de Ciências da Saúde, Federal University of Piaui, Teresina, Brazil.

Noldin, V.F., Isaias, D.B. and Cechinel Filho, V. 2006. Gênero *Calophyllum*: importância química e farmacológica. Quim. Nova 29: 549–554.

Nualkaew, N., Morita, H., Shimokawa, Y., Kinjo, K., Kushiro, T., Deeknamkul, W., Ebizuka, Y. and Abe, I. 2012. Benzophenone Synthase from Garcinia Mangostana L. Pericarps. Phytochemistry 77: 60–69.

Oliveira, C.M.A., Porto, A.L.M., Bittrich, V. and Marsaioli, A.J. 1999. Two polyisoprenylated benzophenones from the floral resins of three Clusia species. Phytochemistry 50: 1073–1079.

Pereira, I.O., Marques, M.J., Pavan, A.L.R., Codonho, B.S., Barbiéric, L., Beijo, L.A., Doriguetto, A.C., D'martín, E.C. and Santos, M.H. 2010. Leishmanicidal activity of benzophenones and extracts from *Garcinia brasiliensis* Mart. fruits. Phytomedicine 17: 339–345.

Pccinelli, A.L., Cuesta-Rubio, O., Chica, M.B., Mahmood, N., Pagano, B., Pavone, M., Barone, V. and Rastrelli, L. 2005. Structural revision of clusianone and 7-epi-clusianone and anti-HIV activity of polyisoprenylated benzophenones. Tetrahedron 61: 8206–8211.

Roberts, J.C. 1961. Naturally occurring xanthones. Chem. Rev. 61: 591–605.

Rodriguez-Amaya, D.B., Kimura, M., Godoy, H.T. and Amaya-Farfan, J. 2008. Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. J. Food Compos. Anal. 21: 445–463.

Rogez, H., Buxant, R., Mignolet, E., Souza, J.N.S., Silva, E.M. and Larondelle, Y., 2004. Chemical composition of the pulp of three typical Amazonian fruits: araça-boi (*Eugenia stipitata*), bacuri (*Platonia insignis*) and cupuaçu (*Theobroma grandiflorum*). Eur. Food Res. Technol. 218: 380–384.

Rokaya, M.B., Munzbergová, Z. and Timsina, B. 2010. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. J. Ethnopharmacol. 130: 485–504.

Rufino, M.S.M., Alves, R.E., Brito, E.S., Pérez-Jimenez, J., Saura-Calixto, F. and Mancini-Filho, J. 2010. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem. 121: 996–1002.

Santos, P.R.P. 2012. Estudos farmacológicos e ensaios pré-clínicos com 1,3-diestearil-2-oleil-glicerol (TG1) derivado de *Platonia insignis* Mart. 95 p. Master's dissertation – Centro de Ciências da Saúde, Federal University of Piaui, Teresina-Brazil.

Santos, P.R.P., Carvalho, R.B.F., Costa Junior, J.S., Freitas, R.M. and Feitosa, C.M. 2013. Survey of physicochemical and pharmacological properties of extracts and compounds isolated from *Platonia insignis* Mart. a perspective for developing phytomedicines. Braz. J. Pharm. 94: 161–168.

Shanley, P., Medina, G. 2005. Frutíferas e Plantas Úteis na Vida da Amazônica. Belém: CIFOR, Imazon.

Santos Júnior, R.Q., Soares, L.C., Maia Filho, A.L.M., Araújo, K.S., Santos, I.M.S.P., Costa Júnior, J.S. and Saffi, J. 2010. Histologic study of skin of wounds healing using the cream of bacuri (*Platonia insignis* Mart.). Conscient. Saude 9: 575–581.

Silva, S. and Donato, H. 1993. Frutas do Brasil. São Paulo, Imprensa de Arte e Projetos e Edições Artísticas, p. 50.

Silva, V.K.L., Figueiredo, R.W., Brito, E.S., Maia, G.A., Sousa, P.H. and Figueiredo, E.A.T. 2010. Stability of the bacuri pulp (*Platonia insignis* Mart.) frozen for 12 months. Sci. Agrotechnol. 34.

Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A. and Petrovick, P.R. 2000. Farmacognosia: Da planta ao medicamento. 2ed. Porto Alegre/Florianópolis. 221–320.

Souza, V.A.B.D., Araújo, E.C.E., Vasconcelos, L.F.L. and Lima, P.S.D.C. 2001. Variability of physical and chemical fruit characteristics of bacury germplasm from mid-north region of Brazil. Braz. Mag. Fruit Cult. 23: 677–683.

Teixeira, G.H.A. 2000. Frutos do bacurizeiro (*Platonia insignis* Mart.): caracterização, qualidade e conservação. 106 p. Master dissertation - Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Universidade Estadual Paulista, Jaboticabal.

Vieira, L.M., Sousa, M.S.B., Mancini-Filho, J. and Lima, A. 2011. Total phenolics and antioxidant capacity "*in vitro*" of tropical fruit pulps. Braz. Mag. Fruit Cult. 33: 888–897.

Villachica, H., Carvalho, J.E.U., Muller, C.H., Diaz, S.C. and Almanza, M. 1996. Frutales y hortaliças promossoras de la Amazônia. Lima, Tratado de Cooperación Amazônica. Secretaria Pró-Tempore. 152–156.

Yamaguichi, K.K.L., Pereira, C.V.L., Lima, E.S. and Veiga Júnior, V.F. 2014. Chemistry and pharmacology of Bacuri (*Platonia insignis*). Sci. Amazon. 3: 39–46.

Weng, J.R., Tsao, L.T., Wang, J.P., W, R.R. and Lin, C.N. 2004. Antiinflammatory phloroglucinols and terpenoids from *Garcinia subelliptica*. J. Nat. Prod. 67: 1796–1799.

Wu, C.C., Lu, Y.H., Wei, B.L., Yang, S.C., Won, S.J. and Lin, C.N. 2008. Phloroglucinols with prooxidant activity from *Garcinia subelliptica*. J. Nat. Prod. 71: 246–250.

Zeisser-Labouebe, M., Lange, N., Gurny, R. and Delie, F. 2006. Hypericin-loaded nanoparticles for the photodynamic treatment of ovarian cancer. Int. J. Pharm. 326: 174–181.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com

Index

Editors' Biography

Professor José L. Martinez

Professor José Luis Martinez is a chemical biologist and did his MSc from the Pontifical Catholic University of Chile. He works at the Vice-Rectory for Research, Development and Innovation of the University of Santiago, Chile. He is the author and co-author of scientific articles in pharmacology and ethnobotany and has a hundred presentations in conferences at national and international scientific events. He is the editor and founder of the Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants (BLACPMA) and is also the Editor of other journals and scientific research books.

Professor Amner Muñoz-Acevedo

Professor Amner is PhD in Chemistry and teaches in the Department of Chemistry and Biology at Universidad of the North Colombia, with wide experience in studies of natural products (plants/microorganisms—volatile/non-volatile secondary metabolites) based on ethnomedicine, organic synthesis, *in vitro* biological assays (antioxidant capacity, cito-toxicity/anticancer, antimicrobial, repellency/insecticidal, antiviral activities), functionalized beverages and food, sustainable uses of agroindustrial wastes, sample preparation techniques (HD, SPME, S-HS, SDE, SFE, SPE, Soxhlet, US, LLE) and instrumental methods of chemical analysis (TLC, GC, LC, MS, NMR, IR, UV/Vis). He has authored/co-authored several papers and delivered lectures at national/international scientific events.

Professor Mahendra Rai

Professor Rai is a senior professor and Basic Science Research Faculty (UGC) at the Department of Biotechnology, Sant Gadge Baba Amravati University, Maharashtra, India. He has been Visiting Scientist in the University of Geneva, Debrecen University, Hungary; University of Campinas, Brazil; Nicolaus Copernicus University, Poland. VSB Technical University of Ostrava, Czech Republic, and National University of Rosario, Argentina. He has published more than 380 research papers in national and international journals. In addition, he has edited/authored more than 45 books and 6 patents.

Taylor & Francis
Taylor & Francis Group
http://taylorandfrancis.com